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Effect of solid retention time on primary sludge prefermentation in the up-flow settler/prefermenter

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ABSTRACT

The study examined prefermentation of primary sludge in an up-flow settler/prefermenter model to enrich wastewater with biodegradable substrate for denitrification. The experiment, preceded by batch tests, was conducted under real conditions in a municipal wastewater treatment plant. The effect of solid retention time (SRT) was the focus while other parameters, such as hydraulic retention time and up-flow velocity, remained constant. The efficiency of chemical oxygen demand (COD) solubilisation and improvement of denitrification potential increased as the SRT increased from 1 to 5 d. At 5 d SRT, dissolved COD and denitrification potential increased by 56 mg/L and 7.6 mg·N–NO_x/L, respectively, corresponding to 35% and 36% of the influent values. However, the onset of intensive biogas production occurred at SRT of 7 d, hindering sludge sedimentation and preventing achievement of a longer SRT.

Keywords: Denitrification potential; Municipal wastewater treatment; Prefermentation; Primary sludge; Up-flow settler/prefermenter

1. Introduction

Advanced nutrient removal is necessary in modern municipal wastewater treatment. In contrast to phosphorus, nitrogen removal is performed exclusively through biological methods. Although alternative processes, such as mainstream anammox [1], show promise, the heterotrophic anoxic denitrification remains the core technology for nitrogen removal and will likely to be for the foreseeable future.

Organic substrate is required for heterotrophic denitrification. If its concentration in the influent wastewater is insufficient, nitrogen will not be removed to the required level. The use of ‘external carbon sources’, such as the addition of methanol, can be very costly and has a number of disadvantages. Alternatively, there is a possibility to use “internal carbon sources”, that is, to generate additional easily degradable substrate from waste or primary sludge [2,3]. Prefermenting the primary sludge can give better results [4].

The predominant techniques for primary sludge prefermentation are side-stream prefermenters or active settlers [5]. There is also a reactor that integrates an up-flow settler and a prefermenter. The wastewater inflow is at the bottom; the settling sludge forms a bed which is continuously elutriated by the upward flow of wastewater. To prevent bed stratification and channelling, sludge can be recirculated or mixed. The primary benefit of this solution is its ability to preferment not only primary sludge, but also the entire influent [6].

A limited number of studies have focused on the use of this type of reactor to improve biological nutrient removal from municipal wastewater [6–8]. It is more commonly investigated as the first stage of an anaerobic municipal wastewater treatment system, referred to as a Hydrolysis Up-flow Sludge Blanket (HUSB) reactor [9].

Solid retention time (SRT) is one of the crucial factors in effective prefermenter operation [10]. The proteins, lipids and carbohydrates present in primary sludge require

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several days to be hydrolysed, that is, converted into soluble products, and an even longer period of time is necessary to convert them into readily degradable short-chain fatty acids (SCFA). However, exceeding a certain time threshold leads to methanogenesis and the loss of hydrolysis and acidification products [10–12]. Despite this, previous research has largely focused on other parameters, such as up-flow velocity or hydraulic retention time (HRT), as the main controlled factors, with SRT being only a resultant value. Therefore, this study evaluates the effectiveness of the up-flow settler/prefermenter in relation to SRT, while keeping other parameters constant. Furthermore, the effect of the prefermenter on denitrification efficiency was assessed directly using denitrification rate and potential tests [13,14], in addition to the indirect approach used by other researchers, for example, through the production of soluble chemical oxygen demand (COD) or SCFA. This pilot study was also carried out directly at a wastewater treatment plant, to consider real-world circumstances, such as variability of wastewater composition. It was preceded by conducting batch tests of primary sludge prefermentation to establish the suitable range of SRT for the main experiment.

2. Materials and methods

Initial sludge prefermentation batch tests were conducted in 2 L glass bottles sealed with ground stoppers and using un-thickened primary sludge. The bottles were incubated at 20°C with the headspace periodically flushed with nitrogen and the contents mixed. Two tests were prepared using sludge obtained by 2-h laboratory sedimentation of raw influent wastewater, whilst the third test used sludge sample taken from the primary sludge pumping station at the "Kujawy" wastewater treatment plant (Kraków, Poland). The sludge was sampled on daily basis (working days) and the soluble (filtered) and coagulated COD of the supernatant were analysed (the latter only in the first two tests). Additionally, the nitrate utilisation rate (NUR) test was performed periodically to assess the COD fractions in the filtered supernatant.

The up-flow settler/prefermenter model (Fig. 1) was located in the coarse screen hall of the "Kujawy" wastewater treatment plant. The model of 0.2 m internal diameter, 2.0 m active height and 63 L active volume of was constructed using a Plexiglass tube. It was equipped with several side ports for sludge sampling and recirculation. Coarse screened raw wastewater (taken directly from the screen channel) was fed continuously into the model from the bottom. A constant up-flow velocity of 0.8 m/h (HRT of 2.5 h) was maintained. The sludge bed which was formed by settled particles was mixed through periodic sludge recirculation (0.08 m^3/h for 15 min every 1.5 h), according to the conclusions drawn by Ligero et al. [7].

It was assumed that the effectiveness of the up-flow settler/prefermenter would be investigated as separate experiment at each planned SRT. Prior to each experiment, the settler/prefermenter was cleaned and then operated for a period equal to the planned SRT, to facilitate sludge bed formation. To achieve steady-state conditions, the settler was run for three times the SRT prior to sampling. Daily removal of an appropriate volume of sludge (1/SRT of sludge bed) was used to control SRT. The SRT was thus considered "nominal", according to Münch et al. [15], that is, the calculation only included the sludge discharge with sludge wastage, and did not take into account the suspended solids (SS) load discharged with the effluent. This approach facilitated SRT control and has also been used by some other researchers investigating prefermenter performance (e.g., [16,17]; possibly by Gonçalves et al. [6] – this paper is not clear on this issue). Moreover, it has also been used to evaluate some full-scale prefermenters [18].

The actual measurement period lasted for subsequent 6 d, with influent and effluent samples taken daily from the model. The composite samples were collected for approximately two times HRT, with effluent sampling delayed for the time corresponding to the HRT. Every sample was analysed for SS and total, soluble (filtered) and coagulated COD. Moreover, denitrification potential tests were carried out on 3 selected days during each experimental period. The temperature within the reactor model was measured daily.

COD was measured using the MPM 3000 Photometer (WTW, Weilheim, Germany) and Merck 14541 cell tests (Merck, Darmstadt, Germany). Total COD was analysed after sample homogenisation, while soluble COD was measured after filtration through 0.45 μm filters. For the assessment of the "truly soluble" COD fraction, coagulated samples were prepared according to Mamais et al. [19], including coagulation with $ZnSO_4$ (pH > 10.5), sedimentation and filtration. Nitrate was measured in accordance with the Polish Standard PN-C-04576-08, using the sodium salicylate colourimetric method. Nitrite was measured using Merck 14776 cell tests (Merck, Darmstadt, Germany). All measurements, except for nitrite, were performed in duplicate.

The NUR test was used to determine the readily and slowly biodegradable COD fractions of the wastewater (S_S and X_S, respectively), as well as the denitrification

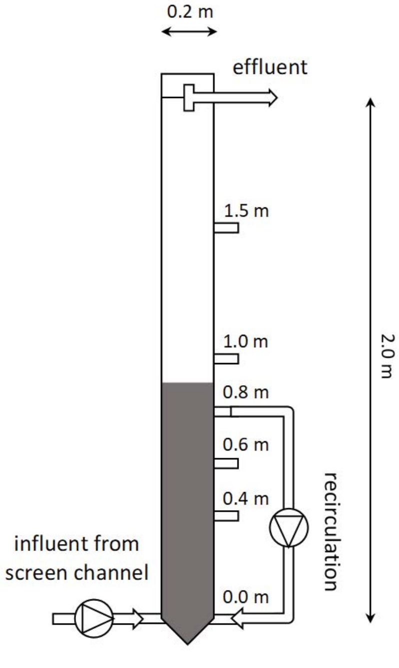

Fig. 1. Schematic representation of the up-flow settler/prefermenter model.

potential. The procedure for this test has been described in detail elsewhere [13,14]. In summary, filtered wastewater was mixed with deoxygenated activated sludge at an S/X_v ratio of 0.03–0.07 g·COD/g·MLVSS, with of 20–30 mg·N–NO$_3$/L (potassium nitrate solution) added. The sludge was mixed in a 1 or 2 L bottle with a ground stopper using a magnetic stirrer at 25°C. Nitrogen was periodically flushed through the headspace. Denitrification progress was monitored by sampling, initially every 10 min and then every 30 min. The samples were immediately centrifuged and the supernatant analysed for nitrate and nitrite. Distinct changes in the course of the decrease in N–NO$_x$ (=N–NO$_3$ + 0.6N–NO$_2$) concentration were used to determine the amount of N–NO$_x$ denitrified using the respective readily and slowly biodegradable wastewater COD fractions and to estimate the respective specific denitrification rates (mg·N–NO$_x$/(g·MLVSS·h)). The readily and slowly biodegradable COD fractions were calculated by assuming an anoxic growth yield coefficient of 0.54 mg·COD$_{\text{biomass}}$/mg·COD$_{\text{substrate}}$ [13,14].

3. Results and discussion

In batch tests with laboratory sedimented sludge (Figs. 2 and 3) there was a marked increase in soluble and coagulated COD, which continued until the 12–14th day of incubation. Following this, there was a decrease in daily increments leading to a plateau in the COD concentration. However, the biodegradable COD fractions analyses demonstrated that their maximum share in generated soluble COD occurred earlier, around the 5th–9th day of incubation,

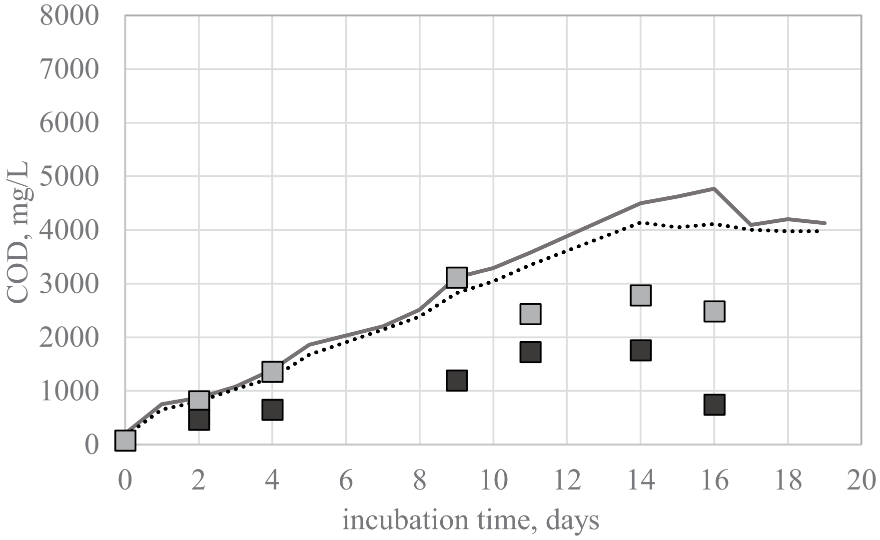

Fig. 2. Results of batch prefermentation test, laboratory sedimented sludge – run 1. Solid line – filtered chemical oxygen demand, dotted line – coagulated chemical oxygen demand. Black squares – rapidly biodegradable chemical oxygen demand (S_S), grey squares – sum of rapidly and slowly biodegradable chemical oxygen demand ($S_S + X_S$).

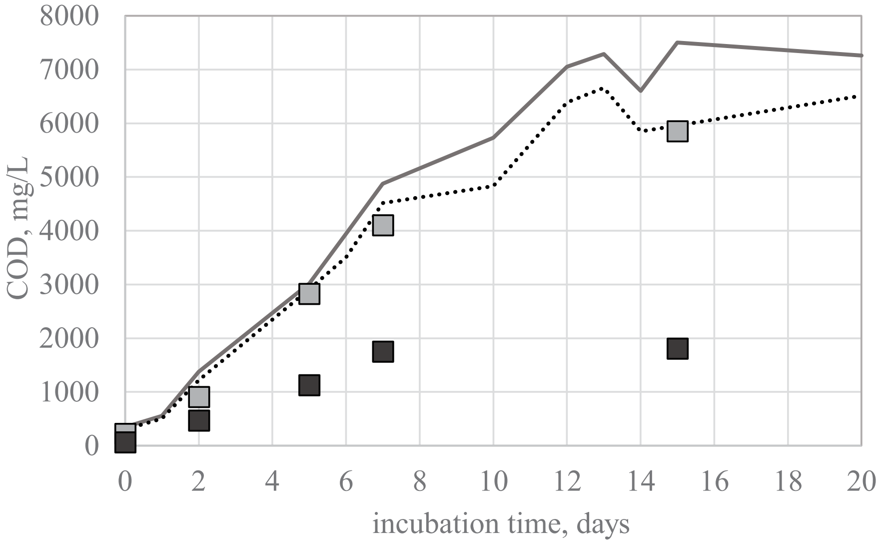

Fig. 3. Results of batch prefermentation test, laboratory sedimented sludge – run 2. Legend – as in Fig. 2.

and was well over 90% for a sum of S_S and X_S. Later, their share decreased significantly, suggesting a higher release of non-biodegradable material.

During the test with the wastewater treatment plant (WWTP) primary sludge (Fig. 4) the increase in soluble COD was considerably reduced and the rapid release phase lasted for only a few days. From 12th day there was a significant decrease in soluble COD, which was an indication of further intensive conversion of organic matter. The highest share of biodegradable COD was reached again on the 5th day of incubation. The different result of this test may be due to the inoculation of the primary sludge with fermentative and methanogenic microorganisms from the sludge processing waters returned into the WWTP inlet and/or prolonged retention of the sludge in the WWTP settling tank hopper.

Based on the findings of the above-mentioned initial tests, it was concluded that the up-flow settler/prefermenter would produce optimal results at an SRT of 5–7 d. Consequently, the experimental SRT range was set at 1–9 d. After discretisation and randomisation, the original research plan included 3, 5, 9, 7 and 1 d SRT experiments. However, observations during the 5 d SRT experiment revealed a random formation of gas bubbles in the sludge bed, indicating start of methane formation. For this reason, 9-d SRT test was excluded and a 7-d test was conducted instead. In the preparation phase of this experiment, considerable gas production led to sludge particles rising and large changes in the height of the sludge bed (90–180 cm). During the actual 7-d SRT experiment, the conditions progressively worsened, resulting in sludge overflow and discharge on the 3rd day. As a result, only a few acceptable measurements were possible for this test. This eventually led to 3, 5, 7 (partly) and 1 d SRT being studied.

Table 1 presents the influent wastewater characteristics during the experiments. In comparison with the typical of municipal wastewater composition [20], it exhibited a moderate to high COD level, with increased SS concentration and related suspended organic matter contamination. There was a significant variability in SS concentration, but this parameter was usually correlated with changes in total COD. However, there was no apparent relationship between total and soluble COD values. Statistical analysis using ANOVA with significance level of 0.05 revealed no significant differences between the influent wastewater parameters of the 1, 3 and 5-d SRT experiments. The mean temperature in the settler for 1, 3, 5 and 7 d SRT experiments was 18.3°C, 21.5°C, 20.9°C and 20.9°C, respectively, with negligible variations.

The removal efficiency of total suspended solids (TSS) in the up-flow settler/prefermenter was high, albeit with considerable variability, most likely due to changes in influent

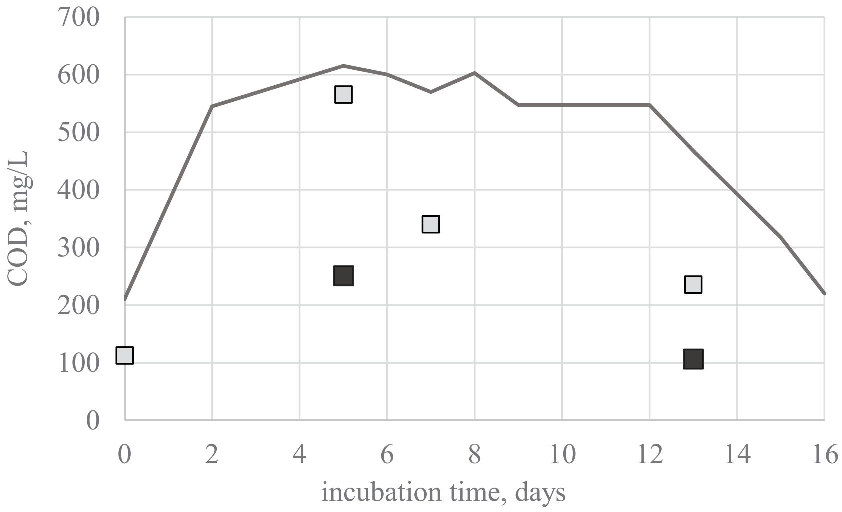

Fig. 4. Results of batch prefermentation test, wastewater treatment plant settled sludge. Legend – as in Fig. 2.

Table 1
Influent wastewater parameters and removal effectiveness for the up-flow settler/prefermenter. Mean and (standard deviation) are given

Solid retention time	1 d	3 d	5 d	7 d*
COD total, mg/L	1,155 (239)	895 (143)	1,059 (303)	1,085 (95)
COD soluble, mg/L	201 (26)	186 (24)	161 (26)	198 (28)
TSS, mg/L	687 (278)	431 (118)	605 (217)	606 (70)
COD removal, %	52 (11)	43 (16)	48 (15)	54 (7)
TSS removal, %	67 (8)	60 (14)	63 (14)	76 (4)

*for 7 d solid retention time results were obtained only for 2 d.

TSS concentration. The average efficiency for 1–5 d SRT was between 60%–67%, which is comparable to that of conventional primary settlers. In the unfinished 7 d SRT experiment, higher values were observed as the high sludge bed probably functioned more efficiently as a filter. However, the limited number of results obtained does not make them reliable.

Comparable TSS removal using the same type of prefermenter has been achieved in other studies with similar values of HRT and up-flow velocity. For example, Gonçalves et al. [6] reported 70% removal at an HRT of 2.8–3.3 h and an up-flow velocity of 0.75–0.9 m/h, while Ligero et al. [7] achieved a 63% removal at an HRT of 2.2 h. On the other hand, conventional types of prefermenters often produce inferior results. For instance, Bouzas et al. [17] obtained only 31%–35% TSS removal for an activated primary settler at an HRT of 2.8 h. Similar observations were reported by Hatziconstantinou et al. [21]. Bouzas et al. [17] found that the results for a side-stream prefermenter were mainly below 50% for SRT of 6 d and below 40% for SRT of 8 d (settler HRT = 2.8 h). This was attributed to the disintegration of sludge flocs at higher SRT levels.

Total COD removal was significant, with an average range of 43% to over 50%, probably due to the increased proportion of suspended COD in the "Kujawy" WWTP influent wastewater. In contrast, Gonçalves et al. [6] reported that only about 25% of the total COD was removed under all conditions.

Table 2 shows the results of the solubilisation of organic matter, measured in terms of soluble and coagulated COD. At SRT = 1 d minimal differences were observed between influent and effluent concentrations, which were considered statistically insignificant (Student's *t*-test for paired samples, with *p*-value of 0.05). Extending the SRT to 3 d resulted in measurable solubilisation (statistically significant): the soluble COD increased by an average of 21 mg/L (11% of the influent value), while the coagulated COD increased by 12.5 mg/L (8%). Prolonging the SRT to 5 d caused an even more substantial increase in soluble COD: an average of 56 mg/L (35% relative to the influent). The corresponding figures for coagulated COD were 30 mg/L and 22%. The high production of soluble and coagulated COD was maintained in the early stages of the uncompleted 7-d SRT experiment.

Gonçalves et al. [6] also found that the up-flow prefermenter exhibited an increase in soluble COD production, from none at 1.2 d SRT to 14.5% at 7.4 d SRT. However, in their study this increase in SRT was accompanied by a simultaneous prolongation of HRT, from 1.1 to 2.8 h, which makes it difficult to distinguish between the influence of each of these parameters separately. Above 7.4 d SRT, there was a decrease in COD solubilization, which the authors attributed to the initiation of methanogenesis. Elefsiniotis [16] reported a significant enhancement in soluble COD and SCFA production in Up-flow Anaerobic Sludge Blanket (UASB) and completely mixed reactors, when the "nominal" SRT was increased from 5 d to 10 d at constant HRT. Only slight improvements were obtained by extending the SRT to 20 d. Bouzas et al. [22] also found that soluble COD and SCFA yield correlated with SRT.

Table 2
Soluble and coagulated chemical oxygen demand in influent and effluent of the up-flow settler/prefermenter for different solid retention time

Solid retention time	Influent COD (mg/L)		Effluent COD (mg/L)	
	Soluble	Coagulated	Soluble	Coagulated
1 d	220	190	230	180
	165	150	185	170
	225	160	220	155
	195	145	185	140
	170	140	175	150
	230	205	240	200
Average	200.8	165.0	205.8	165.8
3 d	200	150	205	160
	142	115	157.5	120
	220	165	235	185
	190	160	225	170
	180	160	210	180
	185	150	210	160
Average	186.2	150.0	207.1	162.5
5 d	155	130	175	160
	205	180	300	220
	160	122.5	180	140
	150	110	230	165
	120	105	160	115
	175	165	255	195
Average	160.8	135.4	216.7	165.8
7 d	170	130	210	190
	225	180	255	230
Average	166.8	137.6	221.1	176.8

The largest increase of soluble COD in this study, both in absolute and relative terms compared to the influent, exceeded that reported by Gonçalves et al. [6], as well as the results reported by other researchers using the same type of up-flow prefermenter [7,23]. Hatziconstantinou et al. [21] achieved a similar relative increase of 35% in relation to the influent in an active primary settler, but at a higher temperature of 24°C. Bouzas et al. [17] reported an even higher relative increase of soluble COD for an active primary settler and a side-stream prefermenter but it is important to note that these results were obtained for sludge with high hydrolysis potential [22].

However, it is noteworthy that Gonçalves et al. [6] used wastewater with much lower concentrations than those in our study, especially for suspended COD fraction. The same observation can be made for the other studies cited. Additionally, the maximum solubilisation reported by Gonçalves et al. [6] was 0.13 mg of produced soluble COD per mg of influent particulate COD, while the average value in our study for the 5 d SRT was only 0.07. One possible explanation for the limited solubilisation could be the different solubilisation potential of the primary sludge. For example, Bouzas et al. [22] noted a twofold difference in solubilisation potential for sludge originating from two different WWTPs. The other likely explanation is that the SRT range used in this study allowed only limited hydrolysis of particulate organic matter. Miron et al. [11] found that SRT > 8d was necessary for protein hydrolysis in primary sludge. Lipid conversion at SRT of 8 d or less was restricted solely to hydrolysis to long chain fatty acids, without further acidification to SCFA. Carbohydrates were the only compounds that underwent hydrolysis and possible further conversion at shorter SRTs, and this trend was intensified with increasing SRT. The shortness of the SRT used in the present study is emphasised by the fact that the assumed "nominal" approach to SRT gives values about 40% higher than the corresponding "actual" SRT in completely mixed reactors used by Miron et al. [11]. Additionally, these researchers conducted experiments at 25°C, which is higher than the 18°C–21°C used in the present study. According to previous research [10] higher temperature is the significant factor in accelerating the rate of the hydrolysis processes.

It was not possible to achieve a longer SRT due to early appearance of intensive gas production, already at an SRT of 7 d. This was a very low value, since Miron et al. [11] reported the onset of methanogenesis at an "actual" SRT of 8–10 d (and at higher temperature). Gonçalves et al. [6] suggested some methanogenic activity only at SRTs above 7.4 d, while Ligero et al. [8] found no biogas production in the up-flow settler/prefermenter although the calculated SRT was over 13 d. Yuan et al. [24] however reported the presence of methanogens already at SRT as low as 7 d in their study on waste sludge prefermentation, due to accidental feeding with digester effluent. Therefore, it seems plausible that the rapid onset of methanogenesis observed in this study was probably due to the discharge of sludge dewatering water to the wastewater inlet at the "Kujawy" WWTP, and thus the inoculation of the primary sludge with methanogens.

Tables 3 and 4 presents the results of denitrification potential and denitrification rates. There was no remarkable enhancement in the total denitrification potential (the sum of N–NO$_x$ denitrified using the readily and slowly biodegradable fractions of COD) at an SRT of 1 d. However, extending the SRT to 3 d led to a rise in the total

Table 3
Denitrification potential of influent and effluent from the up-flow settler/prefermenter for different solid retention time

Solid retention time	Influent denit. potential (mg·N–NO$_x$/L)			Effluent denit. potential (mg·N–NO$_x$/L)		
	Rapid	Slow	Sum	Rapid	Slow	Sum
1 d	12.2	14.5	26.7	12	16.2	28.2
	5.5	14.4	19.9	6.4	15.2	21.6
	10.7	18.7	29.4	11.7	14.3	26
Average	9.5	15.9	25.4	10.0	15.2	25.2
3 d	12	20.8	32.8	7.3	29.7	37
	9.4	13.6	23	12.9	14.1	27
	11.2	17.3	28.5	13.8	18.5	32.3
Average	10.9	17.2	28.1	11.3	20.8	32.1
5 d	9.6	11.1	20.7	13.1	12.8	25.9
	12.8	10.7	23.5	8.6	23.7	32.3
	5.5	13.7	19.2	11.9	15.9	27.8
Average	9.3	11.8	21.1	11.2	17.5	28.7

Table 4
Specific denitrification rates for influent and effluent from the up-flow settler/prefermenter for different solid retention time

Solid retention time	Influent denitrification rate (mg·N–NOx/(g·MLVSS·h))		Effluent denitrification rate (mg·N–NOx/(g·MLVSS·h))	
	Rapid	Slow	Rapid	Slow
1 d	12.3	2.2	12.1	2.5
	3.8	1.7	6.4	1.9
	6.3	2.1	11	2.3
Average	7.5	2.0	9.8	2.2
3 d	6.8	2.1	9.1	3.6
	5.9	1.5	13.1	1.6
	11.3	2.2	7.9	2
Average	8.0	1.9	10.0	2.4
5 d	6.6	1.6	15.1	1.9
	10.5	1.5	9.1	3
	7.8	2.2	13.4	2.4
Average	8.3	1.8	12.5	2.4

denitrification potential of the wastewater in all cases, with an average of 14%, that is, from 28.1 to 32.1 mg·N–NO$_x$/L. The relative increase was even greater for the SRT of 5 d, averaging 36% (from 21.1 to 28.7 mg·N–NO$_x$/L).

With regard to specific denitrification rates, the trends were not so clear, as individual samples showed both increases and decreases to different extents. However, on average, the most significant relative increases were also at 5 d SRT, with a 51% rise for the rapidly degradable fraction (from 8.3 to 12.5 (mg·N–NO$_x$/(g·MLVSS·h)) and 38% rise for the slowly degradable fraction (1.8 to 2.4 mg·N–NO$_x$/(g·MLVSS·h)). This indicates a substantial improvement in both the quantity and quality of the organic substrate for denitrification.

4. Conclusions

The study found that SRT is a very important parameter for the efficient production of additional biodegradable substrate for denitrification from primary sludge in the up-flow settler/prefermenter. Both the COD solubilisation and denitrification potential of wastewater improvement increased with prolongation of SRT from 1-d onwards. The maximal values were achieved at 5 d SRT: 56 mg/L of soluble COD production and 7.6 mg·N–NO$_x$/L increase in the total denitrification potential were the averages of the test series, corresponding to 35% and 36% of the influent wastewater values. A further increase was prevented by intensive gas production at higher SRT, which hindered sludge sedimentation. This was likely the result of methane formation resulting from research carried out under real conditions at a wastewater treatment plant, where sludge could be inoculated by methanogens by returning sludge processing waters. This underlines the differences between results obtained under laboratory conditions and those obtained in a real environment.

References

[1] L. Zhang, L. Jiang, J. Zhang, J. Li, Y. Peng, Enhancing nitrogen removal through directly integrating anammox into mainstream wastewater treatment: advantageous, issues and future study, Bioresour. Technol., 362 (2022) 127827, doi: 10.1016/j.biortech.2022.127827.

[2] H. Wang, C, Jiang, X. Wang, S. Xu, X. Zhuang, Application of internal carbon source: a vital measure to improve nitrogen removal efficiency of low C/N wastewater, Water, 13 (2021) 2338–2350.

[3] Y. Yang, G. Zhao, X. Zhang, J. Du, L. Dong, W. Li, Application of primary sludge fermentation for the production of carbon source for full-scale biological nutrients removal, Pol. J. Environ. Stud., 31 (2022) 4935–4942.

[4] W. Liu, H. Yang, J. Ye, J. Luo, Y.-Y. Li, J. Liu, Short-chain fatty acids recovery from sewage sludge via acidogenic fermentation as a carbon source for denitrification: a review, Bioresour. Technol., 311 (2020) 123446, doi: 10.1016/j.biortech.2020.123446.

[5] W.H. Rössle, W.A. Pretorius, A review of characterisation requirements for in-line prefermenters: Paper 2: process characterisation, Water SA, 27 (2001) 413–422.

[6] R.F. Gonçalves, A.C. Charlier, F. Sammut, Primary fermentation of soluble and particulate organic matter for wastewater treatment, Water Sci. Technol., 30 (1994) 53–62.

[7] P. Ligero, A. Vega, M. Soto, Pretreatment of urban wastewater in a hydrolytic up-flow digester, Water SA, 27 (2001) 399–404.

[8] P. Ligero, A. Vega, M. Soto, Influence of HRT (hydraulic retention time) and SRT (solid retention time) on the hydrolytic pre-treatment of urban wastewater, Water Sci. Technol., 44 (2001) 7–14.

[9] R. Rajagopal, M.R. Choudhury, N. Anwar, B. Goyette, M.S. Rahaman, Influence of pre-hydrolysis on sewage treatment in an up-flow anaerobic sludge blanket (UASB) reactor: a review, Water, 11 (2019) 372–398.

[10] W. Fang, X. Zhang, P. Zhang, J. Wan, H. Guo, D.S. Ghasimi, X.C. Morera, T. Zhang, Overview of key operation factors and strategies for improving fermentative volatile fatty acid production and product regulation from sewage sludge, J. Environ. Sci., 87 (2020) 93–111.

[11] Y. Miron, G. Zeeman, J.B. van Lier, G. Lettinga, The role of sludge retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge in CSTR systems, Water Res., 34 (2000) 1705–1713.

[12] T.H. Duong, K. Grolle, T.T.V. Nga, G. Zeeman, H. Temmink, M. van Eekert, Protein hydrolysis and fermentation under methanogenic and acidifying conditions, Biotechnol. Biofuels, 12 (2019) 1–10.

[13] K. Kujawa, B. Klapwijk, A method to estimate denitrification potential for predenitrifiaction systems using NUR batch test, Water Res., 33 (1999) 2291–2300.

[14] C.M. Lopez-Vazquez, L. Welles, T. Lotti, E. Ficara, E.R. Rene, T.P.H. van der Brand, D. Brdjanovic, M.C.M van Loosdrecht, In: M.C.M. van Loosdrecht, P.H. Nielsen, C.M. Lopez-Vazquez, D. Brdjanovic, Experimental Methods in Wastewater Treatment, IWA Publishing, London, 2016.

[15] E.V. Münch, J. Keller, P. Lant, R. Newell, Mathematical modelling of prefermenters – I. Model development and verification, Water Res., 33 (1999) 2757–2768.

[16] P. Elefsiniotis, The Effect of Operational and Environmental Parameters of the Acid-Phase Anaerobic Digestion of Primary Sludge, Ph.D. Thesis, The University of British Columbia, 1993.

[17] A. Bouzas, J. Ribes, J. Ferrer, A. Seco, Fermentation and elutriation of primary sludge: effect of SRT on process performance, Water Res., 41 (2007) 747–756.

[18] E.V. Münch, F.A. Koch, A survey of prefermenter design, operation and performance in Australia and Canada, Water Sci. Technol., 39 (1999) 105–112.

[19] D. Mamais, D. Jenkins, P. Pitt, A rapid physical-chemical method for the determination of readily biodegradable soluble COD in municipal wastewater, Water Res., 27 (1993) 195–197.

[20] E.I.P. Volcke, K. Solon, Y. Comeau, M. Henze, In: G. Chen, M.C.M. van Loosdrecht, G.A. Ekama, D. Brdjanovic, Biological Wastewater Treatment: Principles, Modelling, Design, IWA Publishing, London, 2020.

[21] G.J. Hatziconstantinou, P. Yannakopoulos, A. Andreakis, Primary sludge hydrolysis for biological nutrient removal, Water Sci. Technol., 34 (1996) 417–423.

[22] A. Bouzas, C. Gabaldón, P. Marzal, J.M. Penya-Roya, A.N.D.A. Seco, Fermentation of municipal primary sludge: effect of SRT and solids concentration on volatile fatty acid production, Environ. Technol., 23 (2002) 863–875.

[23] J.A. Álvarez, E. Armstrong, M. Gómez, M. Soto, Anaerobic treatment of low-strength municipal wastewater by a two-stage pilot plant under psychrophilic conditions, Bioresour. Technol., 99 (2008) 7051–7062.

[24] Q. Yuan, R. Sparling, J.A. Oleszkiewicz, Waste activated sludge fermentation: effect of solids retention time and biomass concentration, Water Res., 43 (2009) 5180–5186.