# Combination of the OSA process with thermal treatment at moderate temperature for excess sludge minimization

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**Abstract**

This study investigated the possibility to couple the conventional Oxic Settling Anaerobic (OSA) process with a thermic treatment at moderate temperature (35 °C). The maximum excess sludge reduction rate (80%) was achieved when the plant was operated under 3 h of hydraulic retention time (HRT). Compared with the conventional OSA system, the thermic treatment enabled a further improvement in excess sludge minimization of 35%. The observed yield coefficient decreased from 0.25 gTSS $gCOD^{-1}$ to 0.10 gTSS $gCOD^{-1}$ when the temperature in the anaerobic reactor was increased to 35 °C, despite the lower HRT (3 h *vs* 6 h). Moreover, the thermic treatment enabled the decrease of filamentous bacteria, thereby improving the sludge settling properties. The thermic treatment enhanced the destruction of extracellular polymeric substances and the increase of endogenous decay rate (from 0.64 $d^{-1}$ to 1.16 $d^{-1}$) that reduced the biomass active fraction (from 22% to 4%).

**Keywords:** Activated sludge; Biomass kinetics; OSA process; Sludge minimization; Thermic treatment.

## 1. Introduction

Nowadays, the disposal of the excess sludge produced in wastewater treatment plants (WWTPs) represents a challenging topic (de Oliveira et al., 2018). In the last decades, the worldwide increase of the WWTP coupled to even more severe regulations on the discharge limits lead to a significant increase of the amount of waste sludge to be disposed (Guo et al., 2013). The waste sludge treatment and disposal represent one of the major operating costs in a WWTP. Indeed, it has been reported that the management and disposal of excess sludge accounts for 25%–60% of the total plant operating costs (Vitanza et al., 2019a). The main options for the excess sludge disposal are three: landfilling, incineration and agricultural reuse. However, all of these create potential risks for the environment and human health (Semblante et al., 2016a). For these reasons, it is important to implement practical solutions aimed at reducing the excess sludge production directly during the wastewater treatment processes.

Among the technologies proposed in the technical literature to obtain sludge minimization, the oxic-settling-anaerobic (OSA) process is one of the most interesting due to its effectiveness, lower operating costs and because its easy implementation (Chudoba et al., 1992; Saby et al., 2003). The OSA process involves the modification of a conventional activated sludge (CAS) plant layout, by inserting an anaerobic reactor in the return activated sludge (RAS) line. Due to the lack of substrate coupled to anaerobic conditions, the uncoupling metabolism is induced, thereby causing the decrease of the biomass growth rate and the excess sludge production (Semblante et al., 2016b). It has been demonstrated that the OSA process might enable sludge reduction rates ranging from 20% to 60%, depending to the hydraulic retention time (HRT) of the anaerobic reactor (Jiang et al., 2018; Sun et al., 2010). In general, long HRTs should be imposed in the anaerobic reactor (> 6 hours) to achieve satisfactory sludge reduction (Ferrentino et al., 2016; Collivignarelli et al., 2017). Indeed, under long anaerobic HRTs, several mechanisms might contribute to the overall reduction of excess sludge production (uncoupling metabolism, endogenous decay, extracellular polymeric substance (EPS) destruction, bacterial lysis and cryptic growth) (Velho et al., 2016; de Oliveira et al., 2018). Nevertheless, a long-term exposure under anaerobic conditions could result in the worsening of the sludge settling properties. Indeed, as reported by previous literature, under prolonged anaerobic conditions the positive effect of the anaerobiosis might be cancelled if simultaneous dissimilatory sulphate reduction occurs. In this case, filamentous bulking caused by presence of filamentous microorganisms like *Thiothrix* is possible (Wanner et al., 1987).

In the previous literature studies, several researchers proposed the combination of the OSA process with physical-chemical methods (ultrasound, ozone, chemical uncouplers etc.), with the aim to improve the sludge minimization while reducing the exposure time under anaerobic conditions (Ye and Li, 2010; Romero-Pareja et al., 2017; Vitanza et al., 2019b;). Among the physical methods applied to achieve excess sludge reduction, the thermal treatment carried out at T <100°C, integrated in the activated sludge stages, causes a significant reduction of excess sludge production, directly linked to an immediate decrease of biological activity and an increase of maintenance energy requirement (Foladori et al., 2010). However, this solution is quite expensive and often revealed to be detrimental for the effluent quality (Wang et al., 2008). Mesophilic conditions might represent a suitable alternative since they are less energy consuming and more stable compared to thermophilic ones, avoiding severe shocks for bacteria while promoting the development of a greater microbial community diversity compared to higher temperature, thereby not affecting the nutrients removal performances (Shi et al., 2018).

To the best authors' knowledge, no studies are available in the literature about the coupling of the OSA process with a thermal treatment at moderate temperature. The thermic-OSA process is supposed to perform a higher sludge reduction efficiency in a smaller anaerobic volume, reducing the negative effect related to a long-term exposure of the activated sludge under anaerobic conditions.

The aim of the present study was to investigate the feasibility to couple a conventional OSA process with a thermic treatment at moderate temperature. A comprehensive comparison between the conventional OSA process and the thermic-OSA process was carried out in this study. The excess sludge reduction and the general performances of the system were monitored and discussed in the light of different operating conditions. More precisely, two different anaerobic HRT (3 hours and 6 hours) in the thermic-OSA configuration were investigated. Moreover, respirometric batch tests were also performed in order to compare the biomass kinetics in the conventional and modified OSA systems.

## 2. Materials and methods

### *2.1 Lab-scale plant layout*

The lab-scale plant used in this study was a CAS system constituted by a plexiglas aerobic tank (22.5 L) and a vertical-flow clarifier (volume of 7.5 L and horizontal surface of $3.14 \cdot 10^{-2}$ $m^2$). The CAS configuration was further modified through the addition of an anaerobic reactor (aluminum) (7.5 L) placed in the RAS line to provide anaerobic conditions in order to realize the OSA configuration. The plant was fed with an acetate based synthetic wastewater with a flow rate of 1 L $h^{-1}$ in continuous mode. The RAS flow was equal to 2.5 L $h^{-1}$ with the aim to reduce the hydraulic retention time of the sludge within the clarifier under oxygen limitation conditions. The RAS was divided into two identical lines that allowed both the recirculation of the sludge to the aerobic reactor and the feeding of the anaerobic reactor with an equal flow. More precisely, the sludge interchange rate was set to 50%. Hence, half of the sludge extracted from the clarifier was recirculated to the aerobic reactor (1.25 L $h^{-1}$), whereas the remaining part was transferred to the anaerobic reactor (1.25 L $h^{-1}$) and further fed by gravity to the aerobic reactor. The aerobic reactor was equipped with an air blower connected with two fine bubbles diffusers that provided dissolved oxygen at a concentration of approximately 2.5 mg $L^{-1}$. The anaerobic reactor was mixed through a magnetic stirrer equipped with a heating element that was used to heat the mixed liquor. The anaerobic reactor was thermally isolated though dense foam robber layers to reduce heat dissipations. The temperature in the anaerobic reactor was continuously monitored through a digital thermometer. The pilot plant was equipped with pH, oxidation-reduction potential (ORP) and dissolved oxygen (DO) probes, which allowed to monitor the system. A schematic layout of the lab-scale plant is depicted in Figure 1.

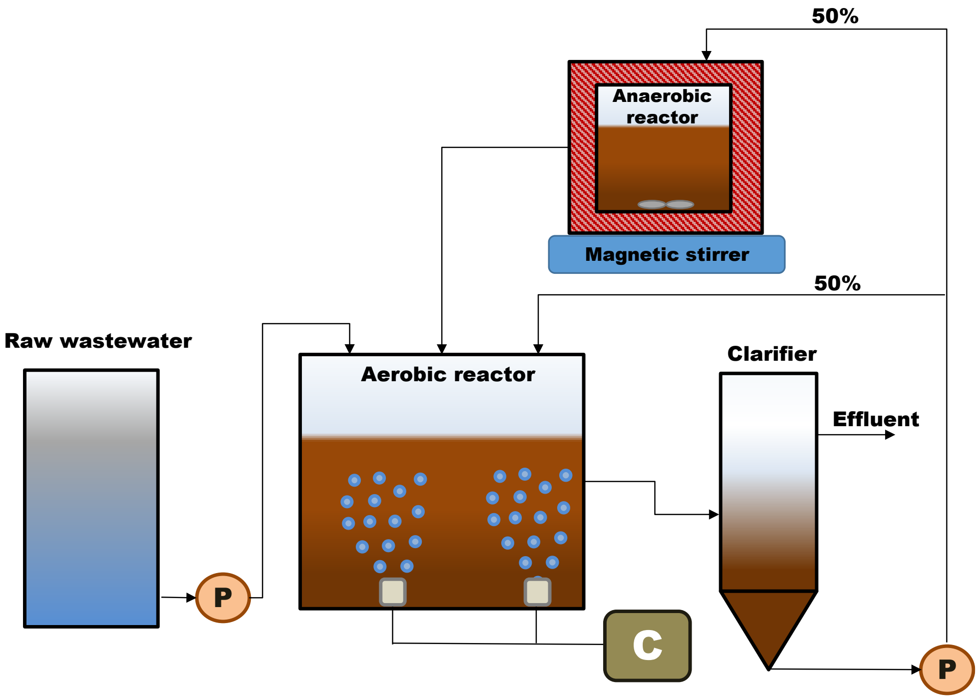

Figure 1: Layout of the lab-scale plant

*2.2 Experimental campaign*

The plant was monitored for 131 days and it was fed with synthetic wastewater during the entire experimental campaign. The synthetic wastewater composition was (in 1 liter of tap water): 1.55g of sodium acetate thihydrate ($CH_3COONa \cdot 3H_2O$), 0.13 g of ammonium chloride ($NH_4Cl$) and 0.056 g of dipotassium phosphate ($K_2HPO_4$). The plant was seeded with activated sludge collected at the municipal WWTP (Acqua dei Corsari, Palermo, Italy).

The experimental campaign was divided into four periods, namely Period 1, Period 2, Period 3 and Period 4. In Period 1 ($1^{st}$ - $46^{th}$ day) the plant was operated according to a (CAS) scheme. Therefore, the RAS flow was entirely recirculated to the aerobic reactor. In Period 2 ($47^{th}$ – $70^{th}$ day), the plant configuration was changed by inserting the anaerobic reactor in the RAS line, hence forming the OSA system. In this period, the anaerobic reactor was maintained at room temperature (T = 15±1 °C) and it was operated with a HRT of 6 hours. In Period 3 ($71^{th}$ – $102^{th}$ day), the plant was operated under the same conditions of the previous period excepting the temperature in the anaerobic reactor that was increased to 35 °C. Lastly, in Period 4 ($103^{th}$ – $131^{th}$ day), the temperature in the anaerobic reactor was maintained at 35 °C, whereas the HRT was decreased to 3 hours by increasing the RAS flow rate from 2.5 L $h^{-1}$ to 5 L $h^{-1}$. In this way, the HRT in the anaerobic reactor was decreased without decreasing its volume and keeping unaltered the sludge interchange rate at 50%. A specific sludge retention time (SRT) was not imposed. In particular, daily sludge withdrawals were carried out with the aim to maintain the total suspended solid (TSS) concentration in the mixed liquor at 3 gTSS $L^{-1}$. Consequently, the SRT was closely related to the biomass growth rate. The temperature of the anaerobic reactor in Period 3 and Period 4 was set at 35 °C with the aim to operate under mesophilic conditions, less energy consuming compared to thermophilic ones, as above discussed. Because the overall volume of the plant increased in Period 2, the organic loading rate (OLR) was slightly increased from Period 2 onward to maintain the same food to microorganism (F/M) ratio of the previous period. The main operating conditions are summarized in Table 1.

Table 1: Operating parameters of the plant

| ***Parameter*** | **Units** | Period 1 | Period 2 | Period 3 | Period 4 |
|---|---|---|---|---|---|
| *Influent flow* | [L $h^{-1}$] | 1 | 1 | 1 | 1 |
| *RAS (from clarifier to aerobic reactor)* | [L $h^{-1}$] | 2.5 | 1.25 | 1.25 | 2.5 |
| *RAS (from clarifier to anaerobic reactor)* | [L $h^{-1}$] | - | 1.25 | 1.25 | 2.5 |
| *HRT of the anaerobic reactor* | [h] | - | 6 | 6 | 3 |
| *Temperature of the anaerobic reactor* | [°C] | - | 15±1 | 35 | 35 |
| *Sludge Retention Time* | [d] | 22 | 33 | 87 | 132 |
| *Biomass concentration* | [gTSS $L^{-1}$] | 3.10±0.12 | 3.0±0.09 | 2.91±0.06 | 2.98±0.04 |
| *Organic Loading Rate* | [kgCOD $m^{-3}d^{-1}$] | 0.57 | 0.60 | 0.61 | 0.60 |
| *Food to microorganisms (F/M)* | [kgCOD $kgTSS^{-1}d^{-1}$] | 0.19±0.02 | 0.20±0.01 | 0.21±0.01 | 0.20±0.02 |

## *2.3 Analytical methods and activated sludge characterization*

The concentration of total and volatile suspended solid (TSS, VSS) in the mixed liquor and the effluent, the chemical oxygen demand (COD), ammonium nitrogen ($NH_4$-N), nitrate nitrogen ($NO_3$-N), nitrite nitrogen ($NO_2$-N) and orthophosphate ($PO_4$-P), were measured according to standard methods (APHA, 2005). Moreover, the main process parameters, including dissolved oxygen concentration, ORP and pH were continuously monitored in the aerobic and anaerobic reactors by means of specific probes connected to a multimeter (WTW 3310).

The content and composition of the EPS was determined by a two-step procedure which involved an extraction phase of the soluble microbial products (SMP) and the exopolymers bounded to the activated sludge flocs (bound EPS) through a thermal extraction process (Le-Clech et al., 2006). Hereafter, the concentration of proteins (PN) and polysaccharides (PS) in each EPS fraction was assessed according to the phenol-sulphuric acid method (DuBois et al., 1956) and by the Folin method (Lowry et al., 1951), respectively. The settling properties of the activated sludge were evaluated by means of the sludge volume index ($SVI_{30}$).

Microscopic observations were carried out for the identification of filamentous bacteria. Observations were performed under phase contrast at 100× and 1000× magnifications. The filamentous microorganisms were morphologically identified using the Eikelboom classification system, whereas the abundance and dominance were estimated according to Jenkins et al. (2003).

### *2.4 Evaluation of biomass growth and kinetics*

The observed yield coefficient was calculated through a mass balance between the sludge production, the sludge wasted and the amount of solids in the effluent, dividing by the cumulated COD removed, according to the literature (Eq. 1) (de Oliveira et al., 2018):

$$Y_{\mathrm{obs}} = \frac{[(X_2 - X_1)V_{\mathrm{r}} + X_{\mathrm{e}}V + X_{\mathrm{s}}V_{\mathrm{s}}]}{(COD_{\mathrm{in}} - COD_{\mathrm{out}})V} \qquad [gTSS\ gCOD^{-1}] \qquad (Eq. 1)$$

where $X_2$ and $X_1$ are the biomass concentrations (g TSS $L^{-1}$) at day (n) and (n-1), $V_r$ is the working volume of the reactor (aerobic + anaerobic), $X_e$ (g TSS $L^{-1}$) is the biomass concentration in the effluent, V is the volume of wastewater treated on a daily base, $X_s$ is the concentration of the waste biomass (g TSS $L^{-1}$), $V_s$ is the volume of waste sludge on a daily base, $COD_{in}$ and $COD_{out}$ are the influent and effluent COD concentration (g $L^{-1}$), respectively.

The kinetic parameters of the heterotrophic biomass, including the endogenous decay coefficient ($b_H$), the net growth coefficient ($\mu_H$), the yield coefficient ($Y_H$), the maximum substrate utilization rate ($v_H$), the specific oxygen uptake rate (SOUR) and the active fraction of the heterotrophic biomass ($f_{XH}$), were assessed by means of respirometric batch tests according to the literature (Capodici et al., 2016).

## 3. Results and discussion

### *3.1 Excess sludge production*

The trend of the observed yield coefficient and the cumulative excess sludge production during the experiment are shown in Figure 2.

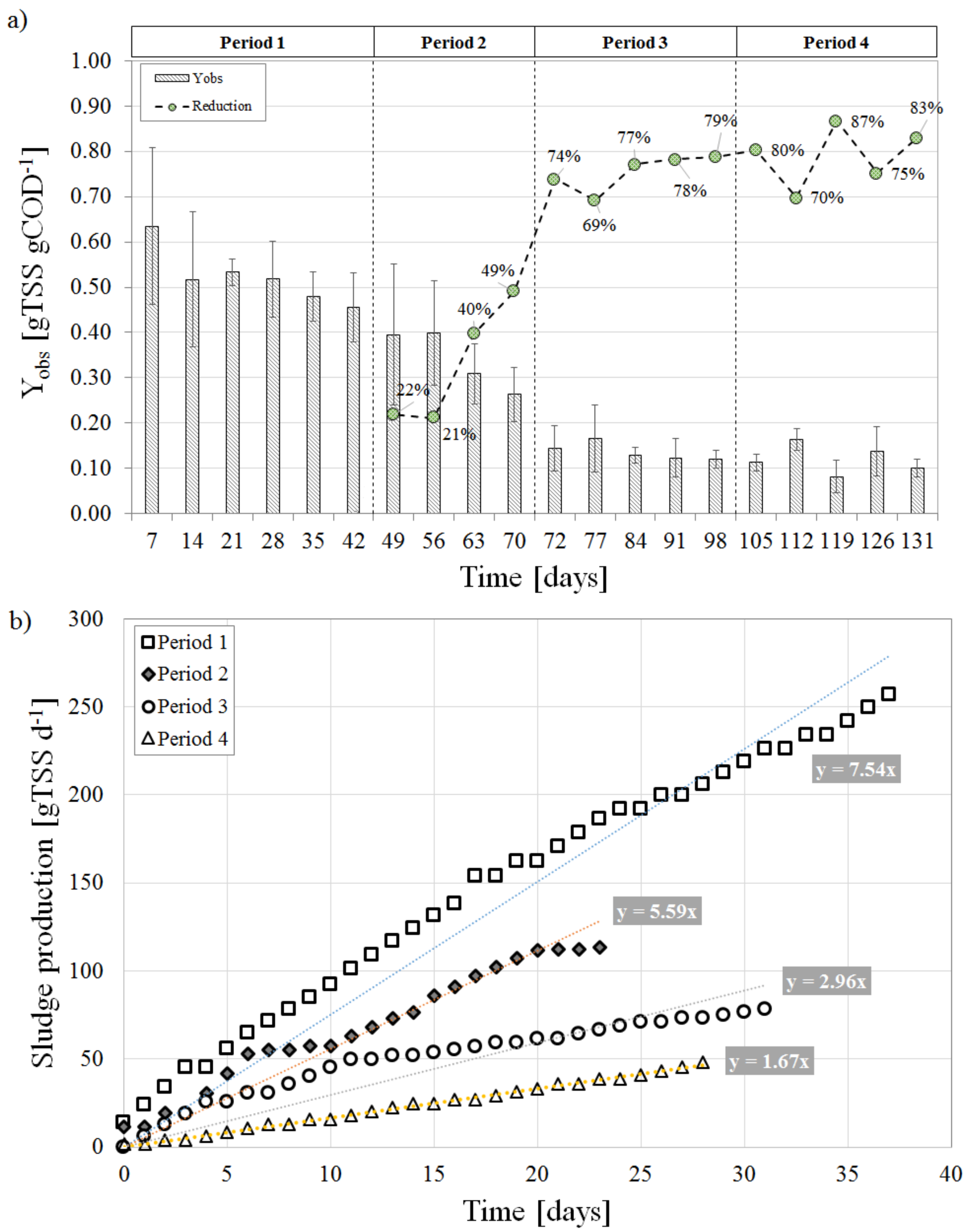


Figure 2: Trend of the observed yield coefficient (bars represent the standard deviation) (a) and cumulative sludge production (b) throughout experiments

The $Y_{obs}$ showed a slight decreased from 0.62 gTSS g$^{-1}$COD to 0.50 gTSS g$^{-1}$COD in Period 1, likely die to biomass acclimation to the new operating conditions of system that characterized by a lower F/M ratio compared with that of the original WWTP from which the sludge was collected. In

Period 2, the $Y_{obs}$ decreased to 0.27 gTSS $g^{-1}$COD, showing an overall reduction rate of 49% related to the OSA implementation. Based on the observed trend of $Y_{obs}$ in Period 2, it is reasonable that steady-state conditions were not achieved, suggesting that a longer observation period would have been necessary. However, because of the significant worsening of the sludge settling properties that occurred in Period 2, the operating conditions were changed before reaching steady state conditions. Nonetheless, the excess sludge reduction and the $Y_{obs}$ values achieved at the end of Period 2 were both comparable with previous studies characterized by OSA operation at room temperature (Coma et al., 2015; Vitanza et al., 2019b). In Period 3, the temperature of the anaerobic reactor was increased up to 35 °C and the $Y_{obs}$ showed a further decrease, reaching a steady value of 0.12 gTSS $g^{-1}$COD at the end of the period. The overall $Y_{obs}$ reduction in Period 3 was 78% (average value) compared to the CAS system and approximately 50% compared to OSA operating at room temperature. This result indicated that the temperature increase in the anaerobic reactor promoted a consistent decrease of the excess sludge production rate. The excess sludge production further decreased in Period 4 when the $Y_{obs}$ remained close to 0.10 gTSS $g^{-1}$COD and the overall reduction efficiency was of approximately 80% compared to CAS configuration.

According to the above results, the cumulative sludge production during the experiment (Fig. 2b) indicated that the maximum rate of sludge production was observed in Period 1, whereas the lowest in Period 4.

The achieved results suggested that the thermic-OSA process enabled a higher efficiency towards the decrease of excess sludge production compared to the OSA process operating at room temperature. Moreover, the role of the HRT in the anaerobic reactor was negligible in the thermic-OSA systems, since the excess sludge reduction rate obtained in Period 3 and Period 4 was comparable although the HRT was halved in Period 4. This result was of significant interest because the thermic-OSA system enabled to achieve a huge reduction of the excess sludge even at lower anaerobic HRT (3 hours) than those required in the conventional OSA system. This allowed operating with a smaller anaerobic volume, thereby reducing the plant footprint and the construction costs for the new facilities. Moreover, the drawbacks related to prolonged anaerobic conditions are reduced, thereby enabling to achieve the excess sludge reduction without affecting the system's performances.

*3.2 Biomass kinetics*

In Table 2, the average values of the main kinetic parameters of the heterotrophic biomass for each experimental period are reported.

Table 2: Average values of the heterotrophic kinetic parameters

| ***Parameter*** | ***Symbol*** | ***Units*** | Period 1 | Period 2 | Period 3 | Period 4 |
|---|---|---|---|---|---|---|
| *Maximum yield coefficient* | $Y_H$ | $[mgTSS\ mg^{-1}COD]$ | 0.57 | 0.38 | 0.31 | 0.27 |
| *Endogenous decay* | $b_H$ | $[d^{-1}]$ | 0.80 | 0.64 | 0.92 | 1.16 |
| *Active fraction* | $f_{XH}$ | $[\%]$ | 17 | 22 | 7 | 4 |
| *Maximum substrate utilization rate* | $v_H$ | $[mgCOD\ mg^{-1}VSS\ d^{-1}]$ | 12.14 | 12.64 | 9.32 | 8.05 |
| *Maximum growth rate* | $\mu_H$ | $[d^{-1}]$ | 6.10 | 5.17 | 3.24 | 2.17 |
| *Maximum specific oxygen utilization rate* | $SOUR_{max}$ | $[mgO_2\ g^{-1}SSV\ h^{-1}]$ | 35.80 | 45.33 | 31.76 | 27.58 |
| *Net growth* | $(\mu_H - b_H)$ | $[d^{-1}]$ | 5.30 | 4.52 | 2.32 | 1.00 |

In Period 1, the achieved values of kinetic parameters were consistent with those of a CAS system as reported in the literature (Metcalf & Eddy, 2014). In Period 2, the maximum yield coefficient ($Y_H$) and the maximum growth rate ($\mu_H$) decreased from 0.57 mgTSS mgCOD$^{-1}$ to 0.38 mgTSS mgCOD$^{-1}$ and from 6.10 d$^{-1}$ to 5.17 d$^{-1}$ respectively, whereas the active fraction increased from 17% to 22%. The $v_H$ and the SOUR increased, suggesting a higher biomass activity in good agreement with the increase of the active fraction. In contrast with was generally observed in previous literature (de Oliveira et al., 2018; Karlikanovaite-Balikci and Yagci, 2019), the $b_H$ decreased despite the implementation of the OSA configuration. Nevertheless, the maximum net growth decreased anyway, indicating that the excess sludge production effectively decreased in Period 2. In Period 3, $Y_H$ decreased from 0.38 mgTSS mg$^{-1}$COD to 0.31 mgTSS mg$^{-1}$COD while the $\mu_H$ reduced from 5.17 d$^{-1}$ to 3.24 d$^{-1}$, indicating that the thermic-OSA process affected the synthesis of new bacterial cells. In this case, the endogenous decay increased from 0.64 $d^{-1}$ to 0.92 $d^{-1}$ in contrast with what observed in the previous Period 2. Therefore, the net growth singificantly decreased by 50% respect to Period 1. In addition, the $v_H$, the $f_{XH}$ and the SOUR decreased, suggesting that the environmental conditions imposed by the thermic-OSA process promoted a significant decrease of biomass activity. The biomass kinetics showed a similar trend in Period 4, indicating that the lower HRT in the anaerobic reactor did not entail a significant modification of biomass kinetics.

The above results indicated that the combination of the thermic treatment with the conventional OSA process involved a significant decrease of the net biomass growth rate, influencing both the synthesis and decay processes. In more detail, the OSA process mainly affected the biomass yield coefficient and the maximum growth rate, whereas the temperature promoted the biomass decay process. Therefore, it is possible to speculate that the OSA process involved a modification of the bacterial metabolism, since the metabolic stress imposed by the lack of substrate under anaerobic conditions decreased the energy availability to convert the organic substrate of the raw wastewater in new biomass. Consequently, the uncoupling metabolism was considered the main factor affecting the excess sludge reduction when the conventional OSA process was implemented.

In contrast, the temperature produced an increase of the endogenous decay rate, likely promoting lysis and decay phenomena as demonstrated by the huge decrease of the active fraction in Period 3 and Period 4 (7 and 4%, respectively). Therefore, among the mechanisms involved in the excess sludge reduction, it is likely that the temperature enhanced mainly the bacterial lysis.

### *3.3 Activated sludge biocenosis*

In CAS systems, the modification of the activated sludge biocenosis is of crucial importance since it could affect the sludge settling properties and therefore the overall nutrients removal performances. Figure 3 depicts the class of abundance of filamentous bacteria during the different experimental periods.

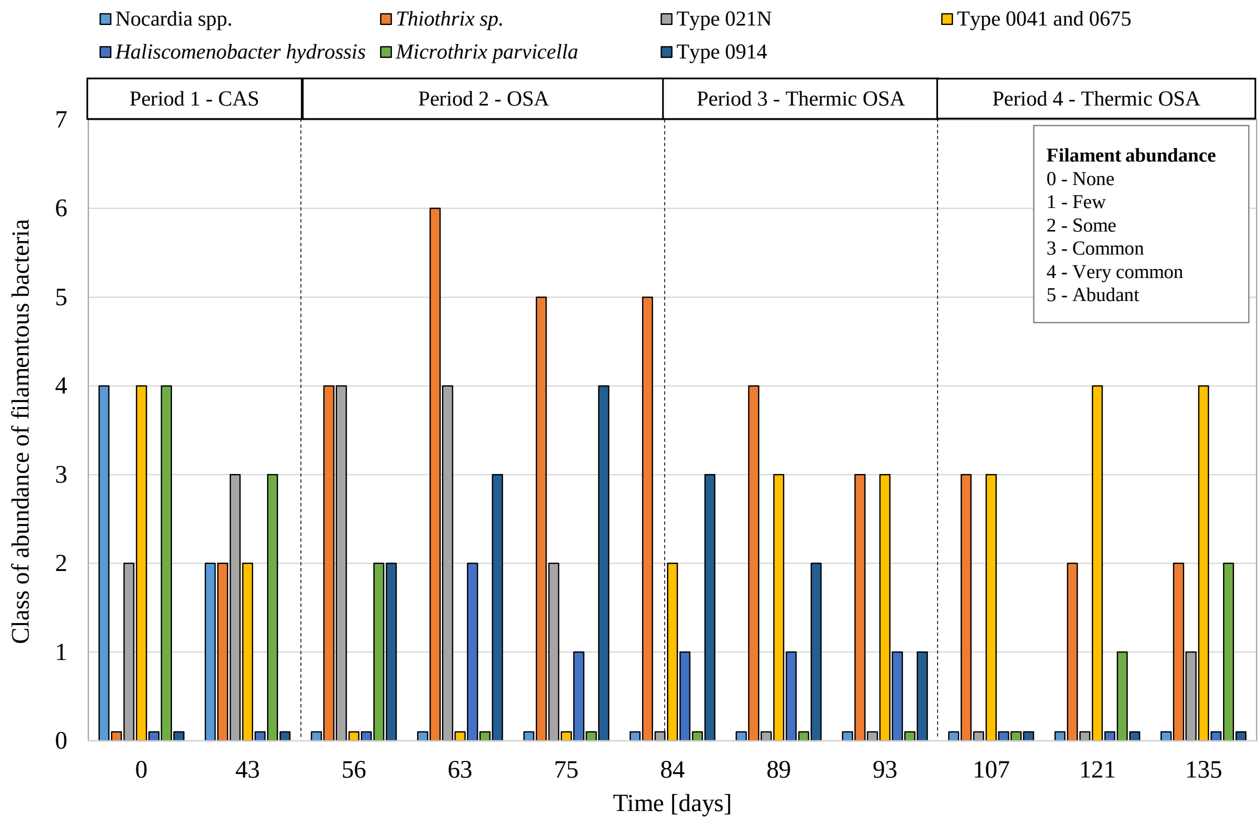


Figure 3: Class of abundance of the main filamentous microorganisms in the activated sludge during the experiment

The dominant filamentous bacteria in the seed sludge were *Microthrix parvicella*, *Nocardia spp*. and type 0041 (class of abundance 4) all of these typical of plants operated under long SRT (Jenkins et al., 2003; Wanner, 2017). At the end of Period 1, the activated sludge biocenosis slightly changed and some filamentous bacteria like type 021N (class 3) and *Thiothrix* (class 2) appeared in the mixed liquor likely because of the use of acetate-based synthetic wastewater (Nielsen et al., 2000), although the dominant filamentous bacteria was still *Microthrix parvicella*.

When the OSA system was started in Period 2, the amount of mixotrophic filamentous bacteria like *Thiothrix* and Type 0914 significantly increased. In particular, the class of abundance of *Thiothrix* reached the maximum value (class 6 – excessive) on 63$^{th}$ day, suggesting that the long-term exposure under anaerobic conditions was favorable for its overgrowth as suggested in the literature (Wanner et al., 1987).

In Period 3, the abundance of mixotrophic bacteria significantly decreased (class of abundance 3) although the HRT in the anaerobic reactor remained the same of Period 2. Moreover, microscopic images (please see supplementary information) allowed observing consistent damages to the cells of *Thiothrix* filaments induced by the high temperature. Therefore, it is reasonable to state that in Period 3 the temperature increase within the anaerobic reactor caused significant damages to the cells of filamentous bacteria contributing to decrease the abundance of *Thiothrix*.

Due to the decrease of the sludge yield coefficient, the SRT increased in Period 3 to 54 days. According to the literature, the maximum growth rate of *Thiothrix* is achieved between 2-20 days of SRT (Jenkins et al., 2003). Therefore, it is possible to speculate that the abundance decrease of *Thiothrix* in Period 3 was due to the increase of both SRT and temperature, although the latter had a greater role since the above-discussed physical damages to the cells induced by the temperature.

In Period 4, the amount of *Thiothrix* still decreased, whereas other types of filamentous bacteria (Type 0675 and Type 0041) were dominant in the activated sludge with a class of abundance of 4. The abundance of these bacterial types increased with the SRT (Jenkins et al., 2003; Wanner, 2017). Indeed, in Period 4 the SRT was increased to more than 70 days because of the further decrease of the sludge yield coefficient and consequently of the excess sludge production.

### *3.4 Sludge settleability and flocs morphology*

The $SVI_{30}$ and the morphology of the activated sludge flocs significantly changed during experiments, according to the different plant configuration and operating conditions (please see supplementary information).

The $SVI_{30}$ of the inoculum sludge was 180 mL $g^{-1}$TSS highlighting the poor settling properties of the activated sludge. In Period 1, the $SVI_{30}$ slightly increased to 230 mL $g^{-1}$TSS indicating that the settling properties of the activated sludge worsened in Period 1, likely due to the different features of the feeding wastewater. Indeed, the use of a readily biodegradable organic substrate instead of real wastewater, might have favored the development of some filamentous bacteria characterized by high affinity toward simple organic molecules like the acetate. The activated sludge flocs were characterized by an average size of approximately 50 μm having an open and weak structure with abundant filaments forming bridges between the same flocs. In Period 2, the $SVI_{30}$ significantly increased during the entire stage, reaching a maximum value of 770 mL $g^{-1}$TSS on day70$^{th}$. This result indicated the occurrence of a severe filamentous bulking due to the significant increase of the abundance in filamentous bacteria as earlier discussed. The size of the flocs decreased to less than 20 μm on average, whereas the number of the interfloc-bridging-filaments dramatically raised. In Period 3, the $SVI_{30}$ decreased during the entire stage reaching a value close to that of the seeded sludge (200 mL $gTSS^{-1}$) on 105$^{th}$ day. Accordingly, the activated sludge flocs appeared bigger (75 μm on average), more compact and with a lower amount of filamentous bacteria. In Period 4, the $SVI_{30}$ remained stably close to 180 mL $g^{-1}$TSS and no significant changes were observed until the end of experiments. Similarly, no significant modifications occurred to the flocs morphology from Period 3 to Period 4, indicating that the change of the operating conditions did not cause substantial modification in the activated sludge flocs.

The results obtained in this study indicated that the implementation of the OSA process caused a dramatic worsening of the sludge settling properties and the occurrence of severe bulking phenomena. This was in contrast with the results reported in many studies related to the OSA system, in which the application of the feasting/fasting regime and the alternation of anaerobic/aerobic conditions produced significant improvements of sludge settling properties (Wang et al., 2015; Rodriguez-Perez and Fermoso, 2016).

Wanner et al. (1987) suggested that the positive effect of the anaerobiosis might be ruined if simultaneous dissimilatory sulphate reduction occurs, because this leads to the growth of filamentous microorganisms like *Thiothrix*. As reported in the literature, a long-term exposure under anaerobic conditions (> 12 hours) promotes the maximum sulphate reduction rate by $SO_4^{2-}$ reducing bacteria (Moon et al., 2015). In the present study, the HRT in the anaerobic reactor was lower than that by Moon and co-authors as favorable to foster the development of mixotrophic bacteria. However, Nielesen et al., 2000) observed the overgrowth of the filamentous sulphur bacterium *Thiothrix* at lower anaerobic HRT (6 hours) likely due to its affinity toward acetate-based synthetic wastewater (Nielsen et al., 2000). Therefore, the use of synthetic wastewater in the present study may have contributed to the *Thiothrix* overgrowth. Nevertheless, it is worth noting that the thermic-OSA process was able to suppress the overgrowth of filamentous bacteria, thereby improving not only the excess sludge reduction performances but also the sludge settling properties. Moreover, as reported in a previous study, the increase of temperature resulted in flocs with a bigger size that improved the settling velocity (Hayet et al., 2010).

### *3.5 EPS content and composition*

Figure 4 shows the trends of the proteins and carbohydrates in the EPS and SMP in the aerobic (Fig. 4a) and anaerobic (Fig. 4b) reactors.

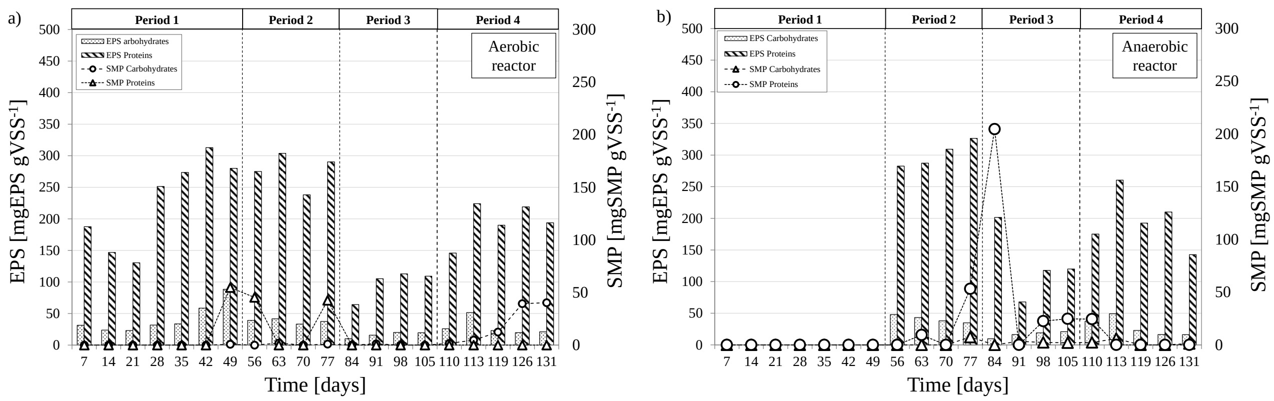


Figure 4: Trends of the proteins and carbohydrates in the EPS and SMP in the aerobic (a) and anaerobic (b) reactors

In Period 1, the amount of EPS (as sum of proteins and carbohydrates) increased from 170 mgEPS $g^{-1}$VSS to 305 mgEPS $g^{-1}$VSS because of the transition from a real wastewater to a synthetic one that may have stimulated the secretion of exopolymers by bacteria. The total EPS content of the activated sludge did not significant change in Period 2 and similar values were observed in both aerobic and anaerobic reactors. In Period 3, the amount of total EPS collapsed in both the aerobic and anaerobic reactors. The total EPS content decreased by approximately 60%, from 300 mgEPS $g^{-1}$VSS to less than 110 mgEPS $g^{-1}$VSS. In Period 4, the EPS content increased reaching a steady value of 200 mgEPS $g^{-1}$VSS.

The SMPs in the aerobic reactor were only sporadically detected during experiments and, apparently, no significant connections with the operating conditions were found. In contrast, the SMP in the anaerobic reactor significantly increased at the beginning of Period 3, when the amount of SMP was comparable to that of the bound EPS (185 mgEPS $g^{-1}$VSS). The increase of SMP in Period 3 was consistent to the decrease of the EPS content, suggesting that the temperature increase caused the deterioration of the polymeric matrix of the sludge, thus resulting in the release of soluble polymers and a consequent increase of its concentration in the bulk liquid.

The amount of proteins was significantly higher than that of carbohydrates that resulted less of 15% of the total EPS content throughout experiments. Nevertheless, also in this case no significant correlations between the EPS composition and the operating conditions were found.

Overall, the implementation of the thermic-OSA process caused a significant decrease of the EPS content in the activated sludge, proportional to the HRT increase in the anaerobic reactor. Overall, in addition to the above-cited mechanisms involved in the excess sludge reduction (uncoupling metabolism and bacterial lysis), the contribution of the EPS destruction was significant especially in Period 3. The above results suggested that the longer is the time of exposure under anaerobic conditions and high temperature, the lower will be the EPS content of the sludge. This could led to a deterioration of the flocs integrity, although this was not observed in the present study. For this reason, it is advisable to operate with low HRT in the anaerobic reactor, since it represents the proper balance to achieve high rates of excess sludge reduction and nutrients removal performances, without significantly affecting the sludge physical properties.

*3.6 Nutrients removal performances*

Figure 5 depicts the trend of the influent and effluent COD concentrations (Fig. 5a), $NH_4$-N (Fig. 5b) and $PO_4$-P (Fig. 5c), as well as the removal efficiencies for each parameter during experiments.

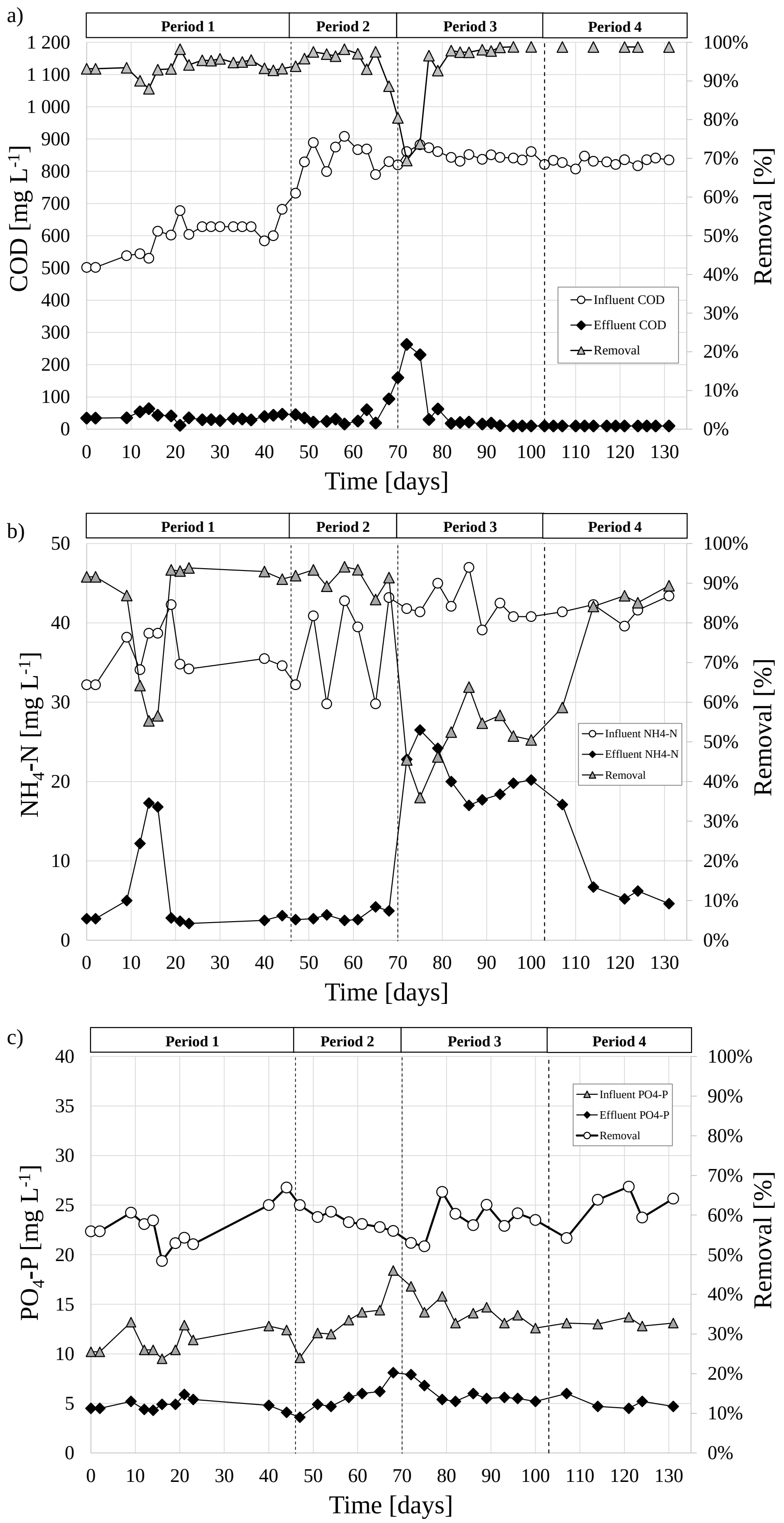

Figure 5: Influent and effluent concentration profiles and removal efficiencies for COD (a), $NH_4$-N (b) and $PO_4$-P (c).

The COD removal was satisfactory during the entire Period 1, when the average removal rate was of 92% and the effluent concentration was constantly below 50 mg $L^{-1}$. The removal rate was also high at the beginning of Period 2 when the OSA configuration was started-up. However, the COD removal efficiency gradually decreased to less than 70% at the end of Period 2. This result was a direct consequence of the worsening of the sludge settling properties that involved a significant loss of TSS with the effluent. Indeed, the soluble COD concentration (evaluated after sample filtration at 0.45 µm - data not shown) was in line with that of the previous Period 1. Therefore, the implementation of the OSA system did not affect the biological COD removal but rather the solid-liquid separation efficiency. In Period 3, when the thermic-OSA process was started, the effluent COD concentration slightly decreased to 40 mg $L^{-1}$ and the COD removal efficiency increased to 97% on average. In Period 4, the COD removal further increased to 99% highlighting that the thermic-OSA process enhanced the biological activity leading to higher COD degradation rate. The different HRT in the anaerobic reactor did not significantly affect the COD removal rate. Long HRT values under anaerobic condition are expected to reduce the biological activity of bacteria, thus the COD removal rate. Indeed, as previously discussed, the implementation of the thermic-OSA process caused a decrease of microbial activity but not high enough to alter the COD removal efficiency. The slight lower COD removal observed in Period 3 compared to Period 4, indicated that a shorter HRT is more appropriate for improving the COD removal rate. Based on the above results, the higher temperature in the anaerobic reactor slightly improved the COD removal efficiency. This result could be explained by the fact that the higher temperature in the anaerobic reactor increased the biomass kinetics, thus stimulating the establishment of more intensive fasting conditions. Once the biomass was recirculated in the aerobic reactor, the higher availability of oxygen and organic substrate led the biomass to a more efficient utilization of the COD. This result confirmed that the more intensive is the fasting phase, the higher is the COD removal efficiency. The $NH_4$-N removal was almost 90% in Period 1, excepting a short period between day 13rd and 16th due to a temporary failure of the aeration system. The same trend was observed in Period 2, when the effluent $NH_4$-N concentration was lower than 2 mg $L^{-1}$, thereby suggesting that the OSA process did not affect the activity of the autotrophic biomass. Nitrification performances significantly decreased in Period 3 to 50% on average when the thermic-OSA system was implemented. The effluent $NH_4$-N concentration remained stable at 20 mg $L^{-1}$ and the contribution of nitrification was very low (<10%). In Period 4, the effluent $NH_4$-N concentrations decreased to 4 mg $L^{-1}$ and the $NH_4$-N removal efficiency raised to 85% on average at the end of the observed period. These results indicated that the activity of the autotrophic biomass was affected by the exposure to both high temperature and extended anaerobic conditions. Indeed, the good removal performances obtained in Period 2 and Period 4 suggested that neither the long anaerobic HRT (Period 2) neither the combination of short anaerobic HRT and high temperature affected the nitrification performances. This result should be taken in careful account especially if the thermic-OSA system is implemented in a plant for biological nutrients removal.

Concerning the $PO_4$-P removal, it was constantly close to 60% throughout experiments. The $PO_4$-P removal was entirely due to the heterotrophic biomass synthesis. Indeed, no $PO_4$-P release was observed in the anaerobic reactor in the different Periods, suggesting that the acclimation of polyphosphate accumulating organisms (PAOs) was not achieved in this study. This result was in contrast with what observed in other studies in the literature referring to OSA systems (Wang et al., 2008; Ye et al., 2008). In fact, the alternation of anaerobic and aerobic conditions typical of OSA system are favorable for the development of PAOs. However, as confirmed by the study carried out by Wang and Park (2002) at temperature higher than 30 °C, the metabolic activity of PAO decreased promoting that of glycogen accumulating organisms (GAOs). Therefore, at high temperature GAOs and PAOs could outcompete for the acetate uptake under anaerobic conditions, thus resulting in poor phosphorous removal efficiency.

*3.7 Possible effects of temperature on excess sludge reduction*

Conventional thermal treatments applied for excess sludge reduction are usually carried out at high temperature (>90°C) (Ødegaard, 2004). Although the high excess sludge reduction rate achievable, the effluent quality is often compromised also entailing very high costs for this implementation. The present study demonstrated that thermic treatment at moderate temperature had positive effects when coupled to OSA process for excess sludge reduction. Indeed, the thermic treatment enhanced the endogenous decay that promoted a consistent decrease of the biomass active fraction. From a kinetic point of view, it is well known that the temperature increase enables a higher biomass activity (Shahzad et al., 2015). Moreover, under anaerobic and prolonged starving conditions, lysis phenomena were promoted and the overall excess sludge minimization increased. Compared with the OSA process operated at room temperature, endogenous decay rates similar to those observed in this study are achievable under prolonged anaerobic conditions that means working with bigger reactors (Karlikanovaite-Balikci and Yagci, 2019). Besides the above discussed negative effects for the activated sludge related to the long duration anaerobic conditions, this affects also the investment costs for the construction of new facilities.

EPS destruction also contributed to sludge minimization. Several studies confirmed that EPS release occurs under anaerobic conditions, with the proteins and polysaccharides that originate from bound EPS are degraded by bacteria (Semblante et al., 2014; Wang et al., 2015). However, an excessive loss of bound EPS could be detrimental for the integrity of the floc structure (Wang et al., 2008). The results obtained in the present study demonstrated that the EPS destruction was enhanced by the temperature increase. Indeed, the amount of EPS decreased of about 50% when the temperature was increased to 35 °C. Considering that a temperature of approximately 80 °C is required to obtain the entire solubilization of the bound-flocs EPS (Le-Clech et al., 2006), it is possible that the temperature investigated in this study enabled only a partial solubilization of the EPS. This allowed obtaining an improvement of sludge reduction, while preserving the sludge flocs integrity.

Moreover, the thermic treatment enabled a significant improvement of the sludge settling properties due to the physical suppression of different types of mixotrophic filamentous bacteria that had caused a severe filamentous bulking when the OSA configuration was operated at room temperature. Our findings demonstrated that the temperature increase to 35 °C caused significant physical damages to the cellular wall of these bacteria and then their progressive disappearance from the system. Longer HRTs in the anaerobic reactor are not advisable in the thermic-OSA process because not providing significant increase of excess sludge reduction rate but rather causing a substantial decrease in the sludge EPS content that could led to deflocculation phenomena and the loss of settling capacity.

Based on these results, the thermic-OSA process allowed achieving a very high excess sludge reduction rate even operating at lower HRT in the anaerobic reactor (Saby et al., 2003; Semblante et al., 2016c). However, it is worth mentioning that the active fraction of the biomass was very low at the end of the experiment. Although this did not affect the nutrients removal performances in this study, likely because the raw wastewater was synthetic hence easily biodegradable, this aspect should be better elucidated in further studies carried out with real wastewater. Indeed, because of the greater complexity of a real wastewater, including also slow biodegradable and particulate organic compounds, the role played by the active fraction might not be negligible as in the present study.

Overall, the main mechanisms involved in the excess sludge reduction in the combined thermic-OSA system acted simultaneously. Consequently, it is rather difficult to discriminate the contribution of each mechanism towards the overall process. Certainly, it can be reasonably assessed that the thermic treatment combined with prolonged anaerobic condition (6 h) significantly enhanced the EPS destruction, which contribution to the sludge reduction was higher compared to the other mechanisms. The contribution of the bacterial lysis increased with the temperature as well and it was considered predominant under moderate HRT in the anaerobic reactor (3 h). Lastly, the contribution of the uncoupling metabolism was maximum when the OSA was operated at room temperature, whereas it decreased, while being not negligible, in the thermic-OSA process.

## Conclusions

The combination of the OSA process with the thermic treatment at moderate temperature (35 °C) enabled a very high efficiency of sludge minimization (80%). The reduction thus achieved was 35% higher than that reported for an OSA process operated at room temperature. The thermic treatment enhanced the destruction of EPS (50%) and the endogenous decay process, thus resulting in a very high excess sludge reduction rate. A significant improvement of the sludge settling properties was also observed because of suppression of many filamentous bacteria driven by the thermic treatment.

## Supplementary information

E-supplementary data of this work can be found in online version of the paper

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**FIGURE CAPTIONS**

Figure 1: Layout of the lab-scale plant

Figure 2: Trend of the observed yield coefficient (bars represent the standard deviation) (a) and cumulative sludge production (b) throughout experiments

Figure 3: Class of abundance of the main filamentous microorganisms in the activated sludge during the experiment

Figure 4: Trends of the proteins and carbohydrates in the EPS and SMP in the aerobic (a) and anaerobic (b) reactors

Figure 5: Influent and effluent concentration profiles and removal efficiencies for COD (a), $NH_4$-N (b) and $PO_4$-P (c).

**TABLE LEGENDS**

Table 1: Operating parameters of the plant

Table 2: Average values of the heterotrophic kinetic parameters