Insights on mechanisms of excess sludge minimization in an oxic-settling-anaerobic process under different operating conditions and plant configurations

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Abstract

In the present research, insights about the mechanisms of excess sludge minimization occurring in an oxic-settling-anaerobic (OSA) were provided. The investigation involved two systems operating in parallel. In particular, a conventional activated sludge (CAS) system as control and a system implementing the OSA process both having a pre-denitrification scheme were considered. Five periods (P1-P5) were studied, during which several operating conditions and configurations were tested. Specifically, the hydraulic retention time (HRT) in the anaerobic reactor of the OSA system (P1 8 h, P2-P3 12 h, P4 8h, P5 12 h) and the return sludge from the anaerobic to the anoxic (scheme A) (P1-P2) or aerobic (scheme B) mainstream reactors (P3-P5) were investigated. The results highlighted that the excess sludge production in the OSA was lower in all the configurations (12-41%). In more detail, the observed yield (Y_{obs}) was reduced from 0.50-0.89 gTSS $gCOD^{-1}$ (control) to 0.22-0.34 gTSS $gCOD^{-1}$ in the OSA. The highest excess sludge reduction (40%) was achieved when the OSA was operated according to scheme B and HRT of 12 hours in the anaerobic reactor (P3). Generally, scheme A enabled the establishment of cell lysis and extracellular polymeric substances (EPS) destructuration, leading to a worsening of process performances when high anaerobic HRT (> 8 h) was imposed. In contrast, scheme B enabled the establishment of maintenance metabolism in addition to the uncoupling metabolism, while cell lysis and EPS destruction were minimized. This allowed obtaining higher sludge reduction yield without compromising the effluent quality.

Keywords: Activated sludge; anaerobic side-stream reactor; biological nutrients removal; excess sludge minimization; oxic-settling-anaerobic (OSA) process; wastewater treatment.

1. Introduction

The conventional activated sludge (CAS) process is the most common system adopted for biological wastewater treatment (Hreiz et al., 2015). This process involves a microbial-mediated conversion of biodegradable organic and nutrient pollutants into gaseous and solids with a residual pollution in the effluent suitable with the environmental requirements for discharge into the receiving water bodies (Wang et al., 2015). Given the large application of the activated sludge process in wastewater treatment, several researches were carried out with the aim to improve its efficiency while minimizing the drawbacks (Cai et al., 2021; Collivignarelli et al., 2019).

Among these, the excess sludge management, including its treatment and disposal operation, is arising as one of the most concern topic of the last decade (Collivignarelli et al. 2019). The excess sludge is a by-product of the activated sludge process that includes the residues of bacterial metabolism as well as inert solids. The amount of excess sludge considerably increased during the last years due to the more stringent environmental requirements, the growth of population and consequent urbanization. For instance, the average excess sludge production in Europe was estimated at approximately 9.7 million tons/year (on dry basis) (Collivignarelli et al., 2019), whereas in China it was close to 60 million tons/year (Cheng et al., 2022). Excess sludge disposal could involve secondary environmental pollution, depending on the disposal practices (e.g., incineration, landfill disposal, etc.). Moreover, the treatment of excess sludge accounts up to 40-60% of the total operating cost in wastewater treatment plants (WWTPs) based on activated sludge process, thereby generating a noticeable economic impact (Arif et al., 2020).

For these reasons, reducing the excess sludge production has become a prominent research challenge. To accomplish this, several researches explored the use of innovative technologies (Di Iaconi et al., 2020; Li and Tabassum, 2022; Sun et al., 2022), whereas others investigated the optimization of already consolidated ones (Ferrentino et al., 2019; 2021). The aim of these technologies is to achieve low production of excess sludge without compromising the removal performance. The main technologies for sludge reduction are based on chemical, mechanical or thermal treatments (Li et al., 2021; Zhang et al., 2021) as well as biological (Ferrentino et al., 2019; Cheng et al., 2022).

Among the biological processes applied for excess sludge reduction, the oxic settling anaerobic (OSA) has received great attention by the scientific and technical communities. Indeed, the OSA process has the advantage of reducing the sludge production in the water line thus reducing the sludge amount to be treated in the sludge line (Di Iaconi et al., 2020). The OSA process involves the modification of a CAS scheme by inserting an anaerobic side-stream reactor (ASSR) in the return activated sludge line (Semblante et al., 2016a). Compared with thermal or chemical technologies, the OSA process is more suitable for retrofitting existing plants (e.g., use of dismissed facilities) and involves lower economic impacts on the plant's operating costs. The performance of OSA processes in terms of sludge reduction could be comparable or even higher, in some cases, to that of physical-chemical technologies. Ferrentino and co-authors obtained up to 69% of sludge reduction by employing a novel process based on a ASSR-like scheme (Ferrentino et al., 2021). In a study carried out on an OSA process coupled with a membrane bioreactor (MBR), the authors obtained 58% of sludge reduction (Fida et al., 2021), whereas a reduction of 49.6% was achieved when an OSA configuration was implemented in a CAS pilot plant (Vitanza et al., 2019).

Excess sludge reduction in the OSA process takes place through the combination of several mechanisms, comprising uncoupling metabolism, sludge decay, extracellular polymeric substances (EPS) destructuration, bacterial predation and selection of slow-growing bacteria (Ferrentino et al., 2021). These mechanisms are usually overlapping and their impact on sludge reduction and process performances (e.g., nutrients removal, sludge settling properties, etc.) seems to be related to the ASSR operating conditions. For instance, Vitanza and coauthors found that sludge decay and EPS destructuration were the main mechanisms when operating with hydraulic retention times (HRT) of 2 h and 3.7 h in the ASSR (Vitanza et al., 2019). Similarly, cell decay and EPS destructuration were observed in a membrane bioreactor (MBR) coupled with an anoxic side-stream reactor operating with 10 hours HRT (Fida et al., 2021). Corsino et al. (2020b) found that when increasing the HRT in the

ASSR from 6 h to 10 h, sludge minimization was mainly driven by endogenous decay, whereas the contribution of the uncoupling metabolism decreased. Nonetheless, Velho and co-authors observed that a 45% increase of the ASSR volume (HRT from 6 h to 10 h) did not promote a further reduction of excess sludge, since it was affected by other factors such as aerobiosis/anaerobiosis alternation (Velho et al., 2016). In most of the above-mentioned studies, negative effects on the effluent quality due to long HRT in the ASSR are reported. Indeed, negative effects on wastewater quality were reported in previous studies, causing the exceeding of the regulatory discharge limits for the effluent (Semblante et al., 2016b; Jiang et al., 2018).

In this context, although the effect of operating conditions on heterotrophic organisms, hence organic matter removal, was widely reported, to the best of authors' knowledge no studies on autotrophic activity is available. Indeed, high retention time under low oxygen availability could alter the fundamental mechanisms of biological nitrogen removal, hindering nitrification (Cantekin et al., 2019). This aspect is of paramount importance in plants where biological nutrients removal is operated. Therefore, the HRT in the ASSR is a key process parameter to achieve excess sludge reduction without compromising the effluent quality. Nevertheless, the knowledge about the relationships between OSA operating conditions and their effects on process performances is still lacking (Guo et al., 2020). Moreover, the relationship between sludge minimization mechanisms and biological activity of autotrophic biomass was not clearly assessed in previous literature so far.

In this light, this study was aimed at increasing the knowledge about the mechanisms occurring in an OSA process operating under different conditions and configurations and their implications in terms of excess sludge reduction and process performances, with special emphasis on autotrophic bacteria. For this purpose, the performances of a pilot plant conceived for biological nutrient removal and coupled with the OSA process were evaluated. Different HRT in the ASSR were investigated and a novel plant layout was explored. The performances of the OSA plant were compared with that of a control reactor without ASSR and fed with the same real municipal wastewater.

2. Materials and Methods

2.1 Pilot plant description

The experiment involved two systems operating in parallel and both configured according to a pre-denitrification scheme (anoxic + aerobic reactors): i) a plant in which an ASSR was inserted in the sludge return line, namely OSA, and ii) a control plant geometrically identical to the OSA but without the ASSR, namely CAS-C (CAS-control). Furthermore, the OSA plant was operated with two layouts that differed for the recirculation line from the ASSR to the mainstream reactors. Specifically, the mixed liquor from the ASSR was returned to the anoxic or the aerobic reactor according to scheme A and scheme B, respectively. Figure 1 depicts the plant layouts.

[Fig.1]

Both plants were fed with a real municipal wastewater collected after the pre-treatment units (screening and grit removal) of a wastewater treatment plant located in Sicily (Italy). The wastewater was stored into a refrigerated tank to prevent biological reactions. Moreover, no primary sludge removal was carried out. Then, the wastewater was pumped to the anoxic reactor of both plants with a constant flowrate of 1.4 L h^{-1}. The anoxic reactor was continuously mixed by means of a mechanical stirrer. The mixed liquor passed by gravity to the aerobic reactor where two porous-stone diffusers placed at the bottom of the reactor and connected to an air blower provided oxygen. The anoxic and the aerobic reactors had the same operating volume, equal to 23.5 L, thereby resulting in an HRT of 16.8 h. From the aerobic reactor, the mixed liquor was fed to a vertical clarifier (16 L) in which the effluent wastewater and the thickened sludge were separated. The sludge was returned to the anoxic reactor and to the ASSR in the CAS-C and OSA systems respectively, with a flowrate of 1.4 L h^{-1} (RAS-1). The ASSR had an operating volume of 11.2 L or 16.8 L, in order to obtain an HRT of 8 h and 12 h, respectively. From the ASSR, the sludge was recirculated to the mainstream reactors of the OSA plant according to scheme A or scheme B respectively.

An internal flow recirculation (RAS-2, 8.4-9.8 L h^{-1}) returned the mixed liquor enriched in nitrate to the anoxic reactor to enhance heterotrophic denitrification. In scheme B, the RAS-2 was increased to 9.8 L h^{-1} to maintain a high biomass concentration in the anoxic reactor of the OSA system. The effluent wastewater was stored into a tank of 33.6 L to have a 24-h average sample before being discharged.

2.2 Experimental campaign

The pilot plants were operated for 152 days and the experimental campaign was divided into five periods, namely Period 1-5. During Period 1 (39 days), the OSA was operated according to scheme A with an ASSR HRT of 8 hours. In Period 2 (35 days), the plant layout was the same of the previous period, whereas the HRT in the ASSR was increased by 50% (12 h), to trigger the anaerobic conditions. In the latter layout, the sludge holding time under anaerobic/anoxic conditions was the maximum during this period since the biomass from the ASSR (HRT of 12 h) was recirculated to the anoxic reactor (HRT of 16.8 h). To stress starvation conditions of microorganisms, in Period 3 (35 days) the OSA layout was changed to scheme B. Compared with scheme A, where the biomass after starved in the ASSR was returned to the anoxic reactor with continuous supply of fresh wastewater, in scheme B microorganisms were subjected to a longer famine period. Indeed, since the carbonaceous substrate availability in the aerobic reactor was significantly lower than in the anoxic reactor, substrate-lack conditions occurred for a longer time equal to the sum of the HRTs in the ASSR and aerobic reactor (28.8 h), while the prolonged dissolved oxygen-deficiency was reduced compared to Period 2. In Period 4 (28 days), the OSA operated according to scheme B, while reducing the HRT in the ASSR to 8 h.

Lastly, to explore the system ability of recovering the conditions of Period 3, in Period 5 (15 days) the HRT in the ASSR was again increased to 12 h, while maintaining the same plant layout (scheme B). The biomass concentration was maintained at approximately 2.5-3 gTSS/L in both OSA and CAS-C plants, by daily purging a known volume of sludge from the aerobic reactor. Consequently, the sludge retention time (SRT) was not controlled and resulted from the mass balance between the biomass growth yield and the amount withdrawn as excess sludge or effluent total suspended solids. Table 1 summarizes the main operating conditions (Tab.1).

[Tab.1]

2.3 Analytical methods

All the chemical-physical analyses, including total and volatile suspended solid (TSS, VSS) concentrations, suspended settleable solids (SSS) concentration, total chemical oxygen demand (TCOD), biochemical oxygen demand (BOD), total nitrogen (TN), ammonium nitrogen (NH_4–N), nitrate nitrogen (NO_3–N), nitrite nitrogen (NO_2–N), total phosphorous (TP) and orthophosphate (PO_4–P) were performed according to standard methods (APHA, 2012). Total suspended solids concentrations were measured in the influent wastewater and in the effluent of both plants as well as in the mixed liquor of each biological reactor. VSS were measured only in the biological reactors. All the other parameters were measured in the influent and effluent samples. Sludge settling properties were assessed by means of the sludge volume index (SVI). The EPS and the soluble microbial products (SMP) were extracted according to the two-step extraction method reported in the literature (Le-Clech et al., 2006) and subsequently characterized in terms of proteins (Lowry et al., 1951) and carbohydrates (DuBois et al., 1956) content.

All the reactors were equipped with on-line sensors; specifically DO, pH and oxidation reduction potential (ORP) probes were connected to a digital portable multimeter device (WTW 3340).

2.4 Assessment of excess sludge production and calculations

The excess sludge production (ΔX) was evaluated as the mass of solids extracted daily, including the treated effluent, samples for analyses and the waste sludge (eq.1).

$$\Delta X = Q_w \cdot x_w + Q_i \cdot x_e \qquad (\text{gSS } d^{-1}) \quad (\text{eq. } 1)$$

being Q_w the volume of sludge discharged from the aerobic reactor daily, x_w the TSS concentration in the same reactor, Q_i the volume of wastewater treated daily and x_e the average TSS concentration in the effluent.

The excess sludge produced by the OSA and CAS-C systems included both primary (ΔX_I) and secondary sludge (ΔX_{II}). Primary sludge included settleable suspended solids in the raw wastewater prior to biological treatment. Conversely, secondary sludge derived mainly from the conversion of the organic and other pollutants into new biomass. The contribution of both primary and secondary sludge on the overall sludge production were assessed separately. Specifically, the primary sludge was determined by measuring the SSS concentration in the influent multiplied by the influent daily flow (eq.2). Consequently, the secondary sludge was determined as the difference between the overall daily sludge production and that of primary (eq.3).

$$\Delta X_I = Q_i \cdot x_{sss} \quad (\text{gSS } d^{-1}) \qquad (\text{eq. } 2)$$

$$\Delta X_{II} = \Delta X - \Delta X_I \quad (\text{gSS } d^{-1}) \qquad (eq. 3)$$

being x_{sss} the concentration of the suspended settleable solids in the influent wastewater.

Moreover, the observed yield coefficient (Y_{obs}) was calculated as the ratio between the cumulative mass of TSS produced and the cumulative mass of COD removed (eq.4).

$$Y_{obs} = \frac{\Delta X}{Q_i \cdot (TCOD_{in} - TCOD_{out})} \quad (gSST\ gCOD^{-1}) \qquad (eq. 4)$$

where COD_{in} and COD_{out} are concentrations (mgCOD L^{-1}) in the influent and effluent, respectively and the other terms have the same above-mentioned.

The sludge retention time (SRT) was calculated as the ratio between the mass of solids in the reactors and the mass of solids extracted daily (including treated effluent, excess sludge and sludge samples for analytic determinations) (eq.5):

$$SRT = \frac{\sum_i^n V_i \cdot x_i}{\Delta X} \quad (d) \qquad (eq. 5)$$

where V_i is the volume of each biological reactor (L), x_i is the TSS concentration inside V_i (gTSS L^{-1}).

2.5 Assessment of heterotrophic and autotrophic kinetic parameters

To address the effects of the operating conditions and layout configurations of the OSA system on the biomass metabolic activity, kinetic parameters of both autotrophic and heterotrophic microorganisms were assessed by means of respirometric techniques. Specifically, the endogenous decay coefficient (b_H), the maximum growth rate (μ_H), the maximum depletion rate of the organic substrate (v_H), the maximum yield coefficient (Y_H) and the active fraction of the heterotrophic biomass (f_{XH}), as well as the maximum growth rate (μ_A) and the maximum yield coefficient (Y_A) of the autotrophic biomass were carried according to literature (Capodici et al., 2016). The respirometric assays were performed by measuring the oxygen utilization rate (OUR) for the consumption of a readily biodegradable substrate (e.g., acetate for heterotrophic and ammonium chloride for autotrophic bacteria) under controlled temperature (20°C) in both the OSA and CAS-C plant once per week.

2.6 Statistical analysis

To assess if changes of the average values of the kinetic parameters and EPS content in the OSA and CAS-C systems were statistically significant, the one-way ANOVA test was employed with a 5% level of significance.

3. Results and discussion

3.1 Wastewater characteristics and main operating parameters

Table 2 reports the average characteristics of the raw wastewater fed to the OSA and CAS-C systems.

[Tab.2]

The average total COD and BOD concentrations were equal to 732 mg L^{-1} and 292 mg L^{-1}, respectively, although the COD values showed a significant higher variability during experiments. This was ascribed to the variability of TSS content in wastewater that increased the amount of the particulate organic matter in some phases of the experimental campaign. Nevertheless, the BOD/COD ratio was close to 0.40, which resulted within the range of typical values for untreated municipal wastewater (Metcalf and Eddy, 2015). The pH and ORP were slightly lower compared with the typical values reported in literature. Negative ORP (< -100 mV) indicated reducing conditions in the raw wastewater and likely the occurrence of fermentative reactions in the sewage system that lowered the average pH values. All the other parameters were in line with typical values for raw municipal wastewater (Von Sperling, 2015).

Table 3 reports the average values of DO, pH and ORP in the biological reactors of the OSA and CAS-C plants.

[Tab.3]

As expected, the dissolved oxygen concentration was zero in the anoxic reactors and the ASSR. Air supplying in the aerobic reactors was provided without imposing a precise DO set point. Therefore, the DO concentration resulted from the balancing between the external oxygen supply and the biomass consumption for metabolic reactions. The average DO concentration in the aerobic reactor of the CAS-C was 7.68 mg L^{-1}, thereby higher than that observed in the OSA in all the experimental periods (Table 3). More precisely, the DO concentration decreased with the HRT increase in the ASSR (P2, P3, P5), and even lower values were achieved when scheme B was implemented (P3, P5). Higher oxygen consumption in the aerobic reactor after implementing the OSA process were reported in the literature (Khursheed et al., 2015; Romero Pareja et al., 2016). According to these studies, prolonged fasting in anaerobic reactor forced bacteria to consume their conserved energy in form of adenosine-triphosphate (ATP). Once returned in the enriched-oxygen environment, the internal energy levels were restored and additional substrate consumption is promoted, thereby leading to an additional oxygen requirement (Semblante et al., 2014; Corsino et al., 2020b).

The pH values were comparable in the biological reactors of both plants. Specifically, lower values (7.29-7.37) were observed in the aerobic reactors due to nitrification, whereas it increased in the anoxic (7.51-7.69) because of denitrification (Metcalf and Eddy, 2015). Similarly, pH in the ASSR was slightly higher than that of aerobic reactor because of biological reduction of the residual nitrates entering with the RAS-1 stream.

Accordingly, the ORP values indicated the existence of reducing (-50 mV < ORP < +50 mV) and oxidative (> +50 mV) environments in the anoxic and aerobic reactors, respectively (Semblante et al., 2014). In the ASSR, the ORP was always below -100 mV, thereby indicating the achievement of anaerobic conditions. Lower values (< -150 mV) were obtained when increasing the HRT in the ASSR (P2, P3, P5), which were similar to those reported in previous studies operating with 100% of sludge recirculation to the ASSR (Coma et al., 2013).

3.2 Performances of excess sludge reduction

The excess sludge was daily purged from the OSA and CAS-C plants to maintain a constant TSS concentration in the mixed liquor of both lines. Since no clarification of the raw wastewater was performed prior to biological treatments, the excess sludge also included the settleable solids in the raw wastewater (primary sludge). Figure 2 shows the trends of cumulative sludge production during each experimental period (a), the contribution of the primary and secondary sludge (b), as well as the observed yield coefficients obtained in the OSA and CAS-C (c).

[Fig.2]

The average daily amount of excess sludge produced in the control plant varied during each experimental period, depending on the raw wastewater characteristics (Fig. 2a). Nonetheless, the excess sludge production in the OSA plant was lower in all the investigated configurations, indicating that sludge minimization was achieved successfully. Specifically, the sludge production was lowered by approximately 12% (P1), 29% (P2), 40% (P3), 26% (P4) and 41% (P5), thereby suggesting that the OSA process resulted in higher or lower sludge reduction efficiencies depending on the different operating conditions and configurations implemented. In more detail, the highest reduction efficiencies were obtained when the OSA plant was operated according to scheme B (Period 3 and Period 5), whereas scheme A (Period 1 and Period 2) determined a lower effect on excess sludge minimization. Therefore, at equal HRT in the ASSR (P2 *vs* P3 and P5), a more extended substrate-deficiency condition determined a greater stressor for biomass than a longer oxygen-lack state. In contrast, by operating under the same layout (P1 *vs* P2 and P3-P5 *vs* P4), a higher HRT in the ASSR enabled to achieve higher sludge minimization.

The primary sludge accounted for approximately 50-60% of the overall sludge production in the CAS-C plant (Fig. 2b). It is worth noting that the percentage of primary sludge in the OSA plant was significantly higher, ranging between 65-95%. Specifically, it is possible to note that the implementation of the OSA process acted mainly on the biological solids production. Indeed, mechanisms of excess sludge minimization occurring in the OSA process, affected the bacteria growth and yield resulting in a lower amount of organic substrate converted into bio-solids (Guo et al., 2020). Therefore, primary sludge production was not significantly affected by the insertion of the anaerobic reactor in the RAS line. Thus, the primary sludge resulted predominant in the excess sludge produced by the OSA plant. To our knowledge, the incidence of primary sludge on excess sludge production in plant implementing the OSA process was not considered so far in previous studies. Indeed, in OSA-related studies carried out with real wastewater, pilot plants were fed with the effluent of primary clarifiers (Ferrentino et al., 2019; Nikpour et al., 2020) or primary sludge was not extrapolated from the overall waste sludge produced (Coma et al., 2015; Velho et al., 2016; Ferrentino et al., 2021). Consequently, focusing on the biological fraction of the excess sludge only, it was noted that the efficiency of the OSA process was very high, reaching a maximum of 95% in Period 3.

To evaluate the excess sludge reduction due to the insertion of the ASSR and provide a thorough comparison with the available literature, the Y_{obs} of the OSA and CAS-C were calculated (Fig. 2c). The observed yield in the CAS ranged between 0.51 gTSS $gCOD^{-1}$ and 0.89 gTSS $gCOD^{-1}$ in agreement with the results reported in plants fed with real wastewater (Coma et al., 2013; Velho et al., 2016). The Y_{obs} slightly decreased in Period 1 to a steady value close to 0.43 gTSS $gCOD^{-1}$. The delay of the observed yield decrease in the OSA plant was attributed to the acclimation of the biomass to the anaerobic/aerobic and feasting/fasting regimes (Fida et al., 2021). The Y_{obs} further decreased to a steady state value of 0.34 gTSS $gCOD^{-1}$ in Period 2. Therefore, the HRT increase in the ASSR when scheme A was implemented produced a Y_{obs} reduction from 30% to 55%. The above results agreed with previous literature. Indeed, Martins et al. (2020) reported a reduction of the observed yield close to 35% (0.33 gTSS $gCOD^{-1}$) by operating with a HRT of 12 in the ASSR, whereas Coma et al. (2013) observed a decrease of the Y_{obs} close to 36% when the interchange ratio was increased to 100% (the same of the present study) and the HRT in the anaerobic reactor was 6 hours (0.32 gTSS $gCOD^{-1}$). Similar findings were reported in a recent study carried out by Vitanza et al. (2020), thus confirming the remarkable effect of sludge reduction achievable with the OSA process. Nonetheless, when the plant layout was changed from scheme A to B, the Y_{obs} reduction increased to 65%. When steady state conditions were reached in Period 3, the observed yield in the OSA was close to 0.20 gTSS $gCOD^{-1}$ (0.60 gTSS $gCOD^{-1}$ in the control), which resulted comparable with the value obtained in a recent study (Ferrentino et al., 2021), although with a lower HRT in the anaerobic reactor (12 h *vs* 5 days). The lower HRT in the ASSR in Period 4 caused a slight decrease of Y_{obs} reduction that was close to 40% on average (steady value of 0.33 gTSS $gCOD^{-1}$). In contrast, when the operating conditions of Period 3 were restored in Period 5, it was achieved a further decrease of the observed yield (0.25 gTSS $gCOD^{-1}$), indicating that the ability of sludge reduction was recovered.

Compared with the available studies carried out with real wastewater on WWTP configured according to BNR schemes (Coma et al., 2015; Romero Pareja et al., 2018; Nikpour et al., 2020; Ferrentino et al., 2021), the results of the present study indicated that the excess sludge reduction was higher when implementing the scheme B. Thus, a double-growth limitation strategy consisting in a prolonged substrate limitation after a sufficient long retention under anaerobic condition, allowed obtaining a higher excess sludge minimization. Indeed, it was likely that the internal ATP restoration in the mainstream reactor was lower in scheme B compared to scheme A, because of the lower organic carbon availability in the aerobic reactor compared to the anoxic (Ferrer-Polonio et al., 2017). Therefore, the lower energy availability deriving from the organic substrate oxidation did not support the ATP restoration, thus reducing the biomass growth. A detailed explanation of these results is provided in the sections below.

3.3 Nutrients removal efficiency

It is important to stress that when processes for sludge minimization are implemented, the effluent quality must not be compromised. For this reason, the effluent wastewater was extensively monitored during the entire experiment. Figure 3 depicts the trends of the total COD (Fig. 3a) and total phosphorous (Fig. 3b), in the influent and effluent wastewater of the OSA and CAS-C plants, as well as the removal efficiencies.

[Fig.3]

The average value of total COD in the influent stream was equal to 732 mg L^{-1}, whereas it ranged between 12 mg L^{-1} and 148 mg L^{-1} in the effluents of both plants. In general, the COD removal efficiency was comparable in the two systems, ranging between 75-95%, thereby highlighting that COD removal was not affected by the OSA process. These results were consistent with those reported by other studies, where no effects on COD removal were observed when the OSA process was implemented (Vitanza et al., 2020; Fida et al., 2021).

Concerning phosphorous removal, the OSA system enabled slightly better performances than CAS-C on average. Indeed, when the HRT in the ASSR was of 12 h (Period 2, Period 3 and Period 5), the mean effluent orthophosphate concentrations of the OSA and the CAS-C were close 9.5 mg L^{-1} and 13.6 L^{-1}, respectively, resulting in removal efficiencies close to 46% and 34%. When the HRT was lower than 12 h, both the plants exhibited similar results, suggesting that phosphorous removal was mainly related to metabolic consumption. These findings were consistent with previous literature, where an increase of P removal was observed in plant implementing the OSA process (de Oliveira et al., 2018; Martins et al., 2020). The authors pointed out that the alternation between aerobic and anaerobic environments might favor the selection of phosphate accumulating organisms (PAOs) or denitrifying phosphate accumulating organisms (DPAOs), which, contributed to the biological phosphorous removal (Romero-Pareja et al., 2017; Fazelipour et al., 2021). Nevertheless, it is worth noting that the SRT increase in the OSA could have lowered the phosphorous removal, since a less sludge amount was purged from the system and P-uptake and release continuously occurred when the sludge cycled between aerobic and anaerobic conditions.

Figure 4 shows the trends of ammonium nitrogen (Fig. 4a), total nitrogen (Fig. 4b) in the influent and effluent wastewater of the OSA and CAS-C plants, as well as the removal efficiencies.

[Fig.4]

High nitrification performances were observed in both the OSA and CAS-C plants in Period 1 (Fig. 4a). The effluent NH_4-N concentration was close to 5 mg L^{-1} on average, without highlighting noticeable differences in the two systems. In contrast, in Period 2 a significant decrease of NH_4-N removal was observed in the OSA. Indeed, the effluent concentration increased up to 20 mgNH_4-N L^{-1}, whereas in the CAS-C it was close to 3 mgNH_4-N L^{-1}. When the OSA layout was changed to scheme B (Period 3), the ammonium nitrogen concentration in the OSA effluent rapidly decreased (< 5 mgNH_4-N L^{-1}) and nitrification was restored in less than 5 days. In the remaining periods (Period 4 and Period 5), no significant changes were noted, and nitrification efficiencies higher than 95% were achieved in both the systems. In previous studies, no relevant negative effects concerning nitrification were observed when ASSR was implemented (Coma et al., 2013; Velho et al., 2016; Wang et al., 2020). On the contrary, some authors reported an improvement of nitrification after inserting the ASSR because of the longer SRT that favored the growth of nitrifiers in the main bioreactor (Nikpour et al., 2020; Fida et al., 2021). Nevertheless, in another study, the authors noted a slight decrease of nitrification efficiency when the OSA process was implemented in a pre-denitrification scheme, due to the anaerobic decay of nitrifying microorganisms in the ASSR (Zhou et al., 2015). In this study, when implementing scheme A, the sludge from the ASSR was returned to the anoxic reactor in the mainstream line. Therefore, when a ASSR is inserted in a A/O layout, a partially loss of nitrification efficiency could occur if the sludge starves for long time under not aerated conditions. Moreover, the results obtained in the present study suggested that the HRT in the ASSR did not affect nitrification in the OSA plant, rather than the prolonged exposure under not aerated conditions. Indeed, when the layout was changed to scheme B and the HRT in the ASSR was maintained at 12 h (Period 3, Period 5), high nitrification efficiencies were obtained.

It is worth noting that in scheme A nitrifiers, after starving 12 h under anaerobic conditions, passed to the anoxic reactor in which the HRT was close to 17 h. The long exposure under not aerated conditions likely hindered the nitrifiers activity. This was confirmed by a recent study in which the authors observed a decrease of nitrification when the not aerated exposure time was increased (L. P. Sun et al., 2020). The long exposure under not aerobic environments might affect the metabolism of nitrifiers, leading to their decay (Corsino et al., 2020b).

The mean value of the influent TN was approximately 74 mg L^{-1}, although it showed a noticeable fluctuating trend (Fig. 4b). The effluent concentration showed a high variability during experiments, although not showing a clear relationship with the operating conditions of the OSA plant. Indeed, in Period 1, TN removal was close to 25% on average in both the OSA and CAS-C, and the main nitrogen form in the effluent was nitrate (> 95%). This was attributed to a low denitrification efficiency due to a low BOD/nitrate ratio (< 3) that limited the nitrates dissimilation in the anoxic reactor of both the systems. Thus, TN removal was ascribed to growth processes. In Period 2, nitrification was the limiting step in the OSA plant, resulting in low TN removal (< 20%) and high concentration in the effluent (> 50 mg L^{-1}). In contrast, the effluent TN concentration in the CAS-C decreased to less than 20 mg L^{-1} when organic carbon was not limiting (65^{th} day onward). At steady state in Period 3, both plants showed the highest TN removal, highlighting that transition from scheme A to B did not affect TN removal. In Period 4 and Period 5, the influent organic carbon was again a limiting factor for denitrification, thereby resulting in high nitrate concentration in the effluent. In contrast with what reported in previous studies, denitrification was not improved by the insertion of the ASSR (Cheng et al., 2022). In general, ASSR contributed to the release of additional carbon source resulting from bacterial cell lysis, providing more organic carbon to sustain denitrification (Cheng et al., 2017). In this study, a noticeable improvement in denitrification was not observed, likely because any significant release of intracellular material occurred in the ASSR during experiments.

3.4 Effect of operating conditions on sludge settling properties and EPS content

To address the change occurred on the sludge physical characteristics, the sludge volume index and the EPS content were periodically measured. Figure 4 shows the change in SVI (Fig. 5a), EPS content (Fig. 5b) and SMP (Fig. 5c) in the two systems.

The SVI in the CAS-C fluctuated between 300-500 mL $gTSS^{-1}$ throughout experiments, highlighting the occurrence of bulking sludge. A possible reason was found in the remarkable presence of filamentous bacteria (Thiothrix sp, and Eikelboom types 021N, 0041 and 0675), which abundance was related to the raw wastewater characteristics, such as low ORP, high soluble COD (Thiothrix and type 021 N) (Wanner, 2017), and relatively high SRT (> 12 d) (Zhang et al., 2019). Nonetheless, the OSA did not involve a further deterioration, but rather a decrease of the SVI in most of the experimental periods was observed. Indeed, during Period 1 and Period 4, the SVI showed a decreasing trend, indicating a noticeable improvement of the sludge settling properties. In Period 3 and Period 5 the SVI was constant in the OSA (300 mL $gTSS^{-1}$ and 100 mL $gTSS^{-1}$) and remained lower than the CAS-C (400 mL $gTSS^{-1}$ and 350 mL $gTSS^{-1}$), on average. A different behavior was instead observed in Period 2, when the sludge settling properties showed a significant worsening tendency. In the present study, the abundance of filamentous bacteria was found lower in the OSA than the CAS-C, in general. Previous studies highlighted the worsening of the sludge settleability when the sludge holding time in the ASSR was increased (Z. Sun et al., 2020). In contrast, other studies emphasized the improvement of settleability when increasing the mass of sludge treated within the ASSR (Chon et al., 2011; Coma et al., 2013).

[Fig.5]

The lower abundance of filamentous bacteria as well as better sludge flocculation observed in the OSA could be due to the establishment of feasting/fasting conditions that promoted the selection of microorganisms with storage ability similarly to selector-like systems (Wanner, 2017). Floc former bacteria under high substrate availability had a competitive advantage over the filamentous since they can use the storage compounds as carbon and energy source for growth. On the other hand, non-storing populations will starve when holding in the ASSR and are washed out from the system (Valentino et al., 2017). Therefore, the OSA system enabled a selection of filamentous bacteria and promoted a better flocculation of the activated sludge, thereby improving the settling properties. Nevertheless, in Period 2 the SVI increased rapidly in the OSA suggesting that operating conditions determined a worsening of the floc structure. Although the SVI increase, the abundance of filamentous bacteria did not change compared with the previous period. Indeed, the higher SVI was related to the decrease of EPS content (Fig. 5b). The EPS content in the sludge of the OSA collapsed from Period 1 to Period 2. In more detail, in Period 2 the EPS content in the aerobic reactor of OSA line was almost halved compared to CAS-C (180 mg $gVSS^{-1}$ *vs* 380 mg $gVSS^{-1}$), whereas in the ASSR it was even lower (50 mg $gVSS^{-1}$). This suggested that EPS destructuration occurred in the ASSR during Period 2 (Fig. 5c). Indeed, in Period 3, despite the same HRT in the ASSR, the change of plant layout to scheme B reduced the exposure time under not aerobic condition and EPS destructuration was minimized. Besides, in Period 4 the decrease of the anaerobic HRT further reduced the EPS destructuration, which resulted comparable with what observed in Period 1. Again, in Period 5 the EPS content in the OSA was close to 240 mg $gVSS^{-1}$ that was slightly lower than CAS-C (280 mg $gVSS^{-1}$). These results were in line with previous studies reporting the occurrence of EPS destruction when the sludge remained for a longer time under anaerobic conditions (Ferrentino et al., 2018; Fida et al., 2021). Moreover, in the present study it was highlighted that the EPS destruction was in good relationship with the increase of the sludge holding time under not aerobic condition. Specifically, when the OSA plant was operated according to scheme A, the SMP content was found the highest (Period 1 and Period 2) (Fig 4c), whereas the SMP significantly decreased when scheme B was adopted from Period 3 onward. EPS promote the formation of the structural framework that is responsible for intercellular adhesion (Semblante et al., 2016c). Therefore, based on the above findings, it could be stated that although EPS destruction is a mechanism responsible for sludge minimization in OSA, it could determine a certain deterioration of process performances.

3.5 Effect of operating conditions on mechanisms involved in excess sludge minimization

The kinetic parameters of heterotrophic and autotrophic biomass were determined and compared with that of the control system in order to gain insights about the effects of the operating conditions implemented in the OSA system. Uncoupling metabolism, cell lysis, maintenance metabolism and EPS destruction were considered as the main sludge minimization mechanisms, thus excluding bacterial predation (Cheng et al., 2021). Bacterial predation was not considered since neither worms, nor carnivorous protozoa or archaea were observed in the OSA system. Each mechanism was associated with the variation of a specific kinetic parameter eventually in combination with another one.

Table 4 summarizes the average values of the heterotrophic and autotrophic kinetic parameters obtained in the OSA and CAS-C during experiments as well as the level of significance (p-value) obtained by comparing the data of OSA and CAS-C between two consecutive periods.

[Tab.4]

According to the statistical analysis, only those parameters for which the p-value was lower than 0.05 were considered affected by the OSA implementation. In Period 1, the only kinetic parameters of the heterotrophic biomass that showed a statistically significant variation were the maximum growth yield (p-value = 0.012) and the maximum COD depletion rate (p-value = 0.021). The endogenous decay and the maximum growth rate in the OSA slightly increased (1.23 d^{-1} *vs* 1.27 d^{-1}) and decreased (3.25 d^{-1} *vs* 2.96 d^{-1}), respectively, although their variations compared to CAS-C were not considered significant (p-values > 0.05). The OUR profiles obtained in the OSA and CAS-C revealed that growth on storage products was negligible (Fig. S1).

[Fig.S1]

Indeed, after oxidation of the external organic substrate that was identified with the maximum OUR values, the biomass respiration rate rapidly decreased to endogenous levels. This indicated that biomass growth did not occur after the external substrate depletion, hence no additional intracellular-stored substrate was available (Ni and Yu, 2008). Moreover, considering the difference between the average EPS content in the aerobic reactor of the CAS-C and that of ASSR (Fig. 5b), it was found that the decrease occurring in the ASSR was statistically significant (p-value = 0.028). Therefore, Period 1 was characterized by a decrease in the conversion yield of substrate into new biomass, without a significant reduction of the maximum growth rate. Indeed, the fraction of the active biomass did not show any significant variation during this period. This result might be explained because the lower biomass yield was balanced by a greater substrate depletion rate (Tab. 4). Previous studies pointed out that alternation of feasting/fasting phases in OSA systems promoted a faster substrate utilization (Karlikanovaite-Balikci and Yagci, 2019; Corsino et al., 2020a). When the sludge after starving in the ASSR is recycled in a substrate and energy-rich environment (anoxic or aerobic reactor), bacteria began feasting on available substrate to replenish energy stocks, leading to high substrate depletion rates. According to Chen et al. (2001), this leads to the occurrence of energy uncoupling. In the same period, a decrease of EPS content with a simultaneous release of SMP in the ASSR, without a simultaneous increase of the endogenous decay rate was noted. Therefore, the increase of the SMP concentration was related to EPS destructuration rather than cell-lysis. Based on these results, two main mechanisms were considered affecting sludge minimization in the OSA system, namely the uncoupling metabolism and the EPS destruction. Indeed, the lower biomass yield was attributed to the less ATP availability of heterotrophic bacteria in the OSA system, thus the energy produced by catabolism was not sufficient to produce new biomass since it was partially used for the internal energy restoration (Chudoba et al., 1992; Semblante et al., 2014). EPS destruction was considered a secondary mechanism, since its percentage reduction was lower than the Y_H.

In Period 2, a noticeable difference between the endogenous decay in CAS-C and OSA was noted (p-value = 0.001) and its increase compared with the previous period was considered statistically significant (p-value = 0.09). Besides, the simultaneous decrease of EPS content (p-value = 0.007) and increase of SMP (p-value = 0.08) suggested that cell lysis occurred in this period. These variations resulted also significant in the OSA compared to the previous period (p-value < 0.05). Similarly, the decrease of the Y_H was considered significant referring to both OSA and CAS-C as well as comparing Period 1 and Period 2 in the OSA system. Nonetheless, its percentage decrease from Period 1 to Period 2 resulted lower compared to the variations of b_H and EPS/SMP content. Therefore, cell lysis and EPS destruction were supposed the main mechanisms affecting sludge reduction in the OSA system, while the role of the uncoupling metabolism was considered secondary. Overall, these mechanisms determined a considerable decrease of the active fraction in the OSA system, which value almost collapsed to approximately 6%.

Period 3 was characterized by a considerable decrease of the maximum growth rate. The difference between the average values observed in the OSA and CAS-C, as well as the increase occurring in the OSA from Period 2 to Period 3 resulted both significant (p-value < 0.05). Among the other parameters, only the Y_H showed a significant reduction, whereas neither b_H nor the EPS content were affected by the novel layout configuration (scheme B) adopted in Period 3. Compared to the previous periods, the OUR profile changed in the OSA and a long tail after the achievement of the maximum respiration rate was noted. High oxygen consumption when external carbon is not available is related to growth on internal storage compounds (Carucci et al., 2001). This behavior was generally observed in systems in which sludge cycled between feasting and fasting conditions (Semblante et al., 2014; Valentino et al., 2017). Moreover, if the sludge loading rate (F/M) is low, the energy available to microorganisms is used for maintenance energy requirements rather than producing new biomass cells (Wei et al., 2003). In Period 3, biomass was subjected to a longer starvation phase compared to the previous period since the sludge was returned in the mainstream aerobic reactor where the availability of organic carbon was lower than the anoxic (Period 2). Therefore, this induced a maintenance metabolism. Furthermore, the uncoupling metabolism was still supposed. Generally, when the sludge from the ASSR is returned to the mainstream reactor, the energy deriving from carbon dissimilation under anoxic (scheme A) or aerobic (scheme B) was used for ATP production which supports bacterial growth. This is expected to be preferential as long as organic matter is available. In scheme B, although the high oxygen availability in the mainstream reactor (aerobic), the lack of carbon did not support the ATP restoration, hence biomass growth was limited. Therefore, in Period 3 it was supposed that maintenance and uncoupling metabolism acted simultaneously.

HRT lowering in the ASSR during Period 4 caused a slight increase of the μ_H and Y_H in the OSA, whereas the other parameters were comparable with that obtained in the previous period. Nevertheless, the increase of the maximum growth rate and yield coefficient in the OSA did not result statistically significant (p-value > 0.05). This result suggested that biomass was already acclimated to a long starvation period, and the decrease of the anaerobic HRT was not enough to determine a significant variation of the kinetic parameters. Similarly, in Period 5 no significant change was noted in the kinetic parameters of the OSA system. Therefore, the difference in sludge minimization obtained in Period 4 and Period 5 could be due to the variation of other operating parameters (e.g., F/M, temperature) affecting the biomass growth.

Referring to the autotrophic biomass, a significant decrease of the biomass yield and the maximum growth rate was observed only during Period 2 and Period 3. Specifically, in Period 2 both the Y_A and the μ_A decreased in the OSA system and these reductions resulted statistically significant (p-value < 0.05) compared to the previous period. In contrast, in Period 3 both parameters increased indicating that the change of the plant layout to scheme B promoted a stress reduction for nitrifiers. These results were in agreement with the nitrification efficiencies observed during Period 2 and Period 3 and confirmed that a long-term exposure under not aerobic conditions determined a reduction of nitrifiers activity.

[Tab.5]

Based on the above considerations, scheme A enabled the establishment of the uncoupling metabolism as reported in previous studies (Chen et al., 2003; Martins et al., 2020). According to other studies, the increase of the anaerobic HRT promoted the onset of bacterial lysis and increased the EPS destructuration (Fida et al., 2021; Sodhi et al., 2021). Consequently, despite about 12-30% of sludge reduction could be achieved, the worsening of nitrification and sludge settling properties represented potential alarming drawbacks in terms of overall system performance. In contrast, scheme B enabled the establishment of the maintenance metabolism in addition to the uncoupling metabolisms, whereas cell lysis and EPS destruction were minimized. In this case, higher sludge reduction yield (26-41%) were obtained, without compromising the effluent quality but also improving specific processes (e.g., P removal).

These findings indicated that scheme B enabled better performances compared to scheme A. Thus, promoting maintenance and uncoupling metabolism allowed avoiding the onset of process dysfunctions (e.g., SVI increase, low nitrification, etc.). At the same time, too high anaerobic HRT is not advisable to avoid the worsening of sludge settling properties.

Conclusions

The excess sludge minimization was studied in an OSA plant fed with real wastewater for 152 days. During the experimental campaign, the operating conditions were changed to evaluate their effects on the process performances and the role of the main mechanisms involved in sludge minimization. The observed yield varied between 0.22 gTSS $gCOD^{-1}$ and 0.34 gTSS $gCOD^{-1}$ in the OSA, showing that lower sludge production was obtained compared to CAS-C (0.50-0.89 gTSS $gCOD^{-1}$). The overall excess sludge production was reduced to a maximum of 40%, whereas the sludge biological fraction decreased by 95% when the OSA was operated according to scheme B and HRT of 12 hours in the ASSR (Period 3). Sludge recirculation from the ASSR to the aerobic reactor (scheme B) promoted the uncoupling metabolism and the maintenance metabolism that enabled high sludge reduction yields without effecting the effluent quality. In contrast, a prolonged exposure under not aerobic conditions (scheme A) resulted in the establishment of cell decay and EPS destruction. This implied lower sludge minimization, worsening of the sludge settling properties and hinder of nitrification.

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Figure captions

Figure 1: Pilot plants layouts

Figure 2: Cumulative excess sludge production in the OSA and CAS-C plants (a); contribution of primary and secondary sludge on excess sludge production (b); trends of the observed yield coefficients and reduction obtained in the OSA and CAS-C.

Figure 3: Influent, effluent concentrations, and removal efficiency of the total COD (a) and total phosphorous (b), in the OSA and CAS-C plants.

Figure 4: Influent, effluent concentrations, and removal efficiency of the ammonium nitrogen (a), and total nitrogen (b) in the OSA and CAS-C plants.

Figure 5: Trends of the SVI in the OSA and CAS-C (a); EPS (b) and SMP (c) content in the biological reactors of OSA (aerobic, ASSR) and CAS-C (aerobic) systems.

Figure S1: Typical OUR profiles of heterotrophic bacteria in Period 1 (a), Period 2 (b) and Period 3, 4, 5 (c).

Table captions

Table 1: Main operating conditions of CAS-C and OSA during the experiment

Table 2: Average characteristics of the influent wastewater

Table 3: Average values of DO, pH and ORP in the anoxic, aerobic, and anaerobic side stream reactor during the experiment in CAS-C and OSA

Table 4: Average values of the heterotrophic and autotrophic kinetic parameters and level of significance obtained from statistical analysis.

Table 5: Summary on the effects of the operating conditions on bacterial metabolism and hypothesis of the main possible mechanisms involved in each period with effects on process performances