

# Effect of Anaerobic Reactor Compartmentalization on Excess Sludge Reduction in ASSR-Based Systems

Sara Mulone\*, Santo F. Corsino, Marco Capodici, Alida Cosenza, Michele Torregrossa

Department of Engineering, University of Palermo, Viale delle Scienze Ed.8, 90128, Palermo, Italy  
[sara.mulone@unipa.it](mailto:sara.mulone@unipa.it)

Excess sludge management represents a major challenge in wastewater treatment, requiring effective minimization strategies. Anaerobic side-stream reactors (ASSR) have emerged as a promising approach to reduce sludge production. The effect of anaerobic reactor hydrodynamics on sludge minimization was evaluated under identical anaerobic exposure time (AET). A laboratory-scale activated sludge system was coupled with a compartmentalized ASSR composed of six reactors in series, approximating plug-flow behavior, and compared with a fully mixed configuration. The compartmentalized system achieved significantly lower sludge yield (Y<sub>obs</sub>), approximately two times lower than the single-compartment reactor at the same AET. This was attributed to the formation of redox gradients, which enhanced microbial stress, endogenous decay, and biomass breakdown. EPS destructurement was observed, with decreasing EPS and increasing SMP, confirming the role of hydrolysis in sludge reduction. Despite a slight decrease in sludge settleability, COD removal remained high. Reactor hydrodynamics was found to play a key role, and compartmentalization proved to be an effective and cost-efficient strategy for sludge minimization.

## 1. Introduction

Excess sludge management represents a major technical, economic, and environmental challenge in wastewater treatment plants, significantly affecting operating costs and process sustainability. The recent EU Directive 2024/3019 highlights the need for efficient sludge management strategies, promoting minimization, resource recovery, and circular economy principles while ensuring environmental protection.

Various sludge reduction techniques have been investigated, including process-based strategies such as extended sludge age, endogenous respiration, side-stream treatments (anaerobic, anoxic, micro-aerobic), aerobic granular sludge systems, membrane bioreactors, and hybrid approaches promoting biomass decay and cell lysis (Collivignarelli et al., 2018). Side-stream reactors, in particular, have shown significant reductions in sludge yield through hydrolysis, decay, and microbial selection mechanisms. Micro-aerobic processes further enhance sludge reduction via increased cell lysis and ATP dissipation (Morello et al., 2022).

Among these technologies, anaerobic side-stream reactors (ASSR) have gained attention as cost-effective and easily retrofittable solutions, achieving sludge reduction of 20–60% (Ferrentino et al., 2021). Traditionally, ASSR performance has been evaluated using HRT and SIR; however, these parameters often fail to fully explain sludge reduction efficiency.

The anaerobic exposure time (AET), recently introduced, integrates exposure duration and frequency, showing a stronger correlation with sludge reduction (Mulone et al., 2024). Despite these advances, the role of reactor hydrodynamics and internal gradients, such as ORP, remains poorly understood. Low ORP conditions may enhance microbial stress and biomass decay (Coma et al., 2013).

Therefore, this study investigates the effect of different hydrodynamic regimes on sludge minimization at equal AET, focusing on the impact of ORP gradients on process performance.

## 2. Materials and methods

In this study, a laboratory-scale plant configured according to a conventional activated sludge (CAS) system was used. The plant consisted of an aeration tank with a variable volume ranging from 17.5 to 22.5 L and a 14.5-L vertical-flow clarifier, connected by gravity. The aerobic reactor was equipped with two porous-stone diffusers connected to an air blower providing a constant airflow of 5 L/min, maintaining a dissolved oxygen concentration of approximately 2 mg/L. To prevent poorly mixed zones, a vertical impeller was installed. The clarifier was equipped with a peristaltic pump that continuously extracted settled sludge from the hopper and recirculated it to the aeration tank at a constant flow rate of 2 L/h. The treated effluent was collected in a tank designed to retain suspended solids eventually escaping from the clarifier, allowing for a more accurate solids mass balance. The ASSR configuration included an anaerobic reactor in line with the sludge recirculation (RAS), through which the entire recirculated sludge stream passed (SIR = 100%). The ASSR consisted of a rectangular tank with a total working volume of 12 L, divided into six compartments of equal volume (2 L each). Each compartment was equipped with an individual impeller to ensure adequate mixing. The transfer of mixed liquor from one compartment to the next occurred through submerged openings located at the bottom of the internal baffles. These openings were offset but symmetrically arranged with respect to the longitudinal axis of the reactor, in order to prevent the formation of preferential flow paths and to promote a plug-flow-like hydrodynamic behaviour. The overall observation period lasted 100 days and was divided into two consecutive phases of 50 days each. Two different AET of 8 and 10 h/day were tested. The results obtained were compared with those previously reported in the literature by Mulone et al. (2024), in which the same operating conditions were applied to an anaerobic reactor characterized by a completely mixed hydrodynamic.

The plant was fed with a synthetic medium at a constant flow rate of 2 L/h. The use of a synthetic influent allowed for precise control of operational parameters (e.g., food-to-microorganism ratio – F/M) when varying the aerobic reactor volume. Moreover, it ensured that the results were not influenced by the qualitative characteristics of the influent. For details on the composition of the synthetic medium, refer to the literature (Corsino et al., 2020).

Figure 1 illustrates a schematic layout of the plant.

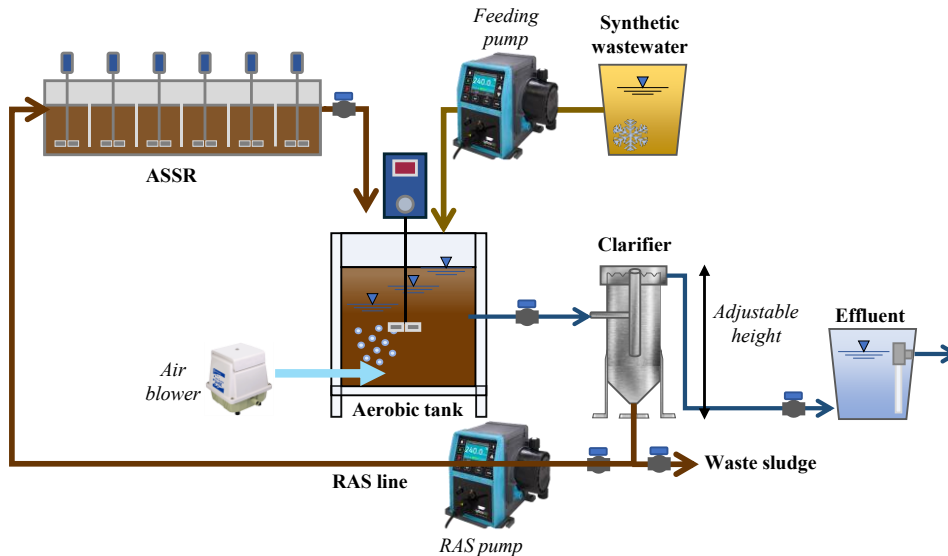


Figure 1: Layout of the experimental reactors.

To maintain the same F/M ratio, the COD load in the synthetic medium was increased to reach 0.35 kgCOD/kgTSS·d, and the nutrient dosage (nitrogen and phosphorus) was increased proportionally to maintain a constant carbon-to-nitrogen-to-phosphorus ratio.

The effectiveness of the ASSR in terms of excess sludge minimization was assessed using the observed yield coefficient ( $Y_{obs}$ ). This was calculated by dividing the cumulative mass of total suspended solids (TSS) produced ( $\Delta X$ ) by the cumulative mass of chemical oxygen demand (COD) removed (eq. 1).

$$Y_{obs} = \frac{\text{mass of TSS produced}}{\text{mass of COD removed}} = \frac{\Delta X}{Q_i \cdot (COD_{in} - COD_{out})} \left[ \frac{gTSS}{gCOD} \right] \quad (1)$$

Where the  $Q_i$  is the daily volume of wastewater treated, and  $COD_{in}$  and  $COD_{out}$  are the influent and effluent, respectively.

All the physical-chemical analyses were performed according to Standard Methods (APHA, 2005), including COD, TSS and sludge volume index (SVI) to assess sludge settling characteristics. Extracellular polymeric substances (EPS) were quantified using colorimetric assays, with proteins expressed as bovine serum albumin equivalents and polysaccharides expressed both as D-glucose equivalents.

Before starting the experiment, a hydrodynamic test was performed on the ASSR using a concentrated solution of sodium chloride (electrical conductivity 40 mS/cm) as a tracer. The NaCl solution was fed continuously with a flow rate of 2 L/h. The trend of the electrical conductivity in each compartment was measured by a dedicated electrode (KLE 325, WTW).

### 3. Results and discussion

#### 3.1 Hydrodynamic behaviour of the ASSR

Figure 2 shows the results of the hydrodynamic test carried out on the ASSR using sodium chloride as a tracer.

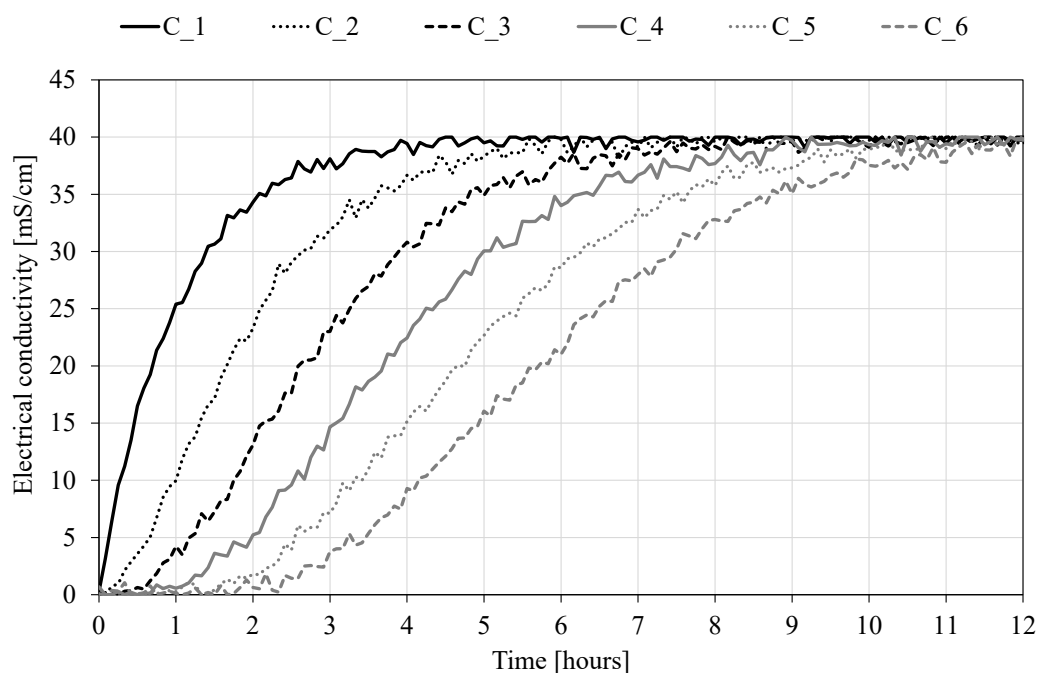


Figure 2: Tracer test results in the ASSR showing electrical conductivity profiles in the six compartments

In each compartment, electrical conductivity increases rapidly and then gradually approaches a stable plateau, indicating that each compartment behaved as a completely mixed reactor with efficient local mixing. The smooth shape of the curves and the absence of sharp peaks suggest that tracer dispersion within each compartment was dominated by mixing rather than by advective transport. The progressive time delay observed from  $C_1$  to  $C_6$  reflects the serial arrangement of the compartments and confirmed the sequential transfer of the mixed liquor through the reactor. This behaviour demonstrated that, although complete mixing is achieved at the compartment scale, the series configuration of the six units resulted in an overall hydrodynamic regime that closely approximates plug-flow conditions. Such a configuration allowed the potential establishment of longitudinal gradients along the reactor length while limiting internal short-circuiting, which could be particularly relevant for processes in which controlled exposure times and redox gradients play a key role.

#### 3.2 Effect of ASSR compartmentalization on $Y_{obs}$ reduction

Figure 3 reports the average value of the  $Y_{obs}$  obtained in this study, compared to those reported in Mulone et al. (2024). The figure shows that, when operating according to CAS layout, the observed yield coefficient was similar for both configurations, with values of approximately 0.69–0.71 gTSS/gCOD, indicating comparable baseline biomass production. When the ASSR was introduced, the  $Y_{obs}$  decreases markedly in both cases. In the 1-compartment (1-C) configuration, the  $Y_{obs}$  decreases to about 0.56 gTSS/gCOD at AET = 8 h/d and to around 0.41 gTSS/gCOD at AET = 10 h/d. In contrast, the 6-compartment configuration exhibited substantially lower  $Y_{obs}$  values at the same AET, reaching approximately 0.29 gTSS/gCOD at AET = 8 h/d and 0.22

gTSS/gCOD at AET = 10 h/d. Thus, at equal AET, reactor compartmentalization led to nearly a twofold reduction in  $Y_{obs}$  and, consequently, in excess sludge production. Another relevant outcome was that, in the compartmentalized configuration, the additional benefit of increasing AET from 8 to 10 h/d was less pronounced than in the completely mixed reactor. This suggested that plug-flow-like hydrodynamics enhanced sludge reduction efficiency even at lower AET values, allowing better performance at moderate anaerobic exposure compared to completely mixed systems operated at higher AET. A possible explanation was that compartmentalization promoted the formation of longitudinal redox and substrate gradients, increasing microbial stress and decay rates locally, while limiting biomass adaptation through continuous mixing. As a result, the selective pressure exerted by anaerobic exposure becomes more effective, reducing the need for longer AET to achieve comparable or improved sludge minimization. These results indicated that, in addition to AET, reactor hydrodynamics played a significant role in controlling biomass yield and excess sludge production.

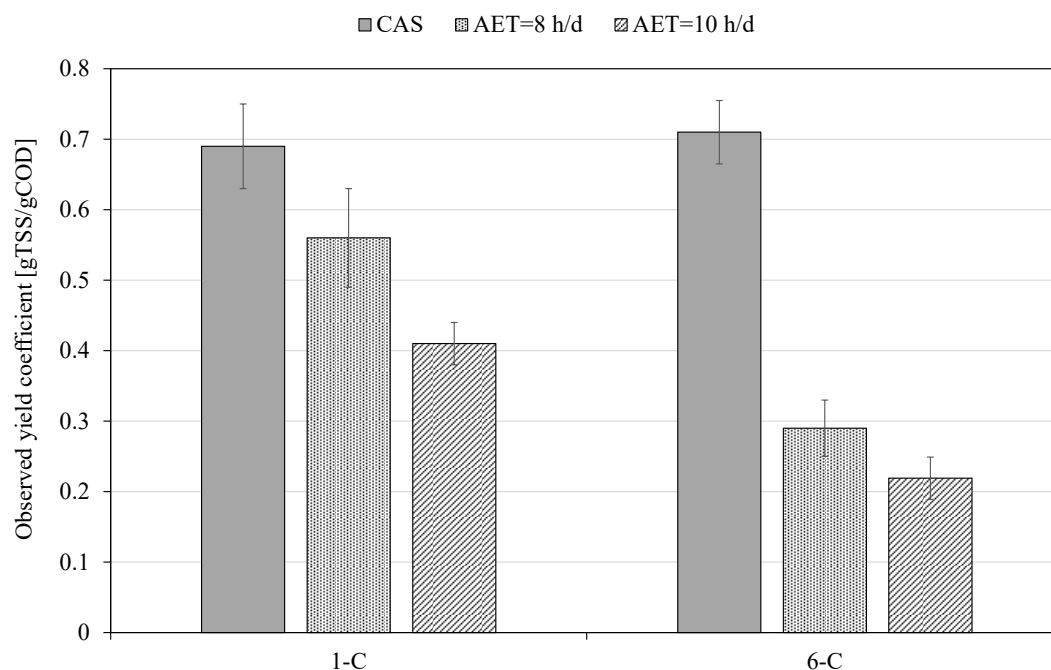


Figure 3: Average values of the  $Y_{obs}$  obtained in this study (6-C) compared with those reported by Mulone et al. (2024) (1-C)

Additional ORP measurements conducted within the anaerobic reactor revealed the establishment of a clear redox gradient along the longitudinal axis of the system. Specifically, ORP values decreased progressively from  $-192.4$  mV in the first compartment to  $-311.7$  mV in the final compartment.

This marked shift toward more reducing conditions of the ASSR can be attributed to the progressive depletion of residual electron acceptors and the concomitant intensification of strictly anaerobic metabolic pathways. In ASSR configurations, such strongly reducing environments are known to promote endogenous decay, cell lysis, and cryptic growth mechanisms (Mulone et al., 2024). Under these conditions, biomass underwent extended exposure to substrate-limited and highly reducing environments, leading to increased maintenance energy requirements and partial mineralization of intracellular storage compounds.

The progressively lower ORP values observed in the final compartment indicated enhanced anaerobic degradation processes, which are directly associated with reduced net biomass yield. The establishment of a pronounced redox gradient supported the hypothesis that prolonged exposure of return sludge to highly reducing conditions was a key mechanism allowing for further reduction in excess sludge production.

### 3.3 Mechanisms of sludge minimization: EPS destructuration

Figure 4 illustrates the longitudinal evolution of the bound EPS and soluble microbial products (SMP) along the six completely mixed compartments of the compartmentalized ASSR, compared with the concentration measured in the aerobic reactor (AE).

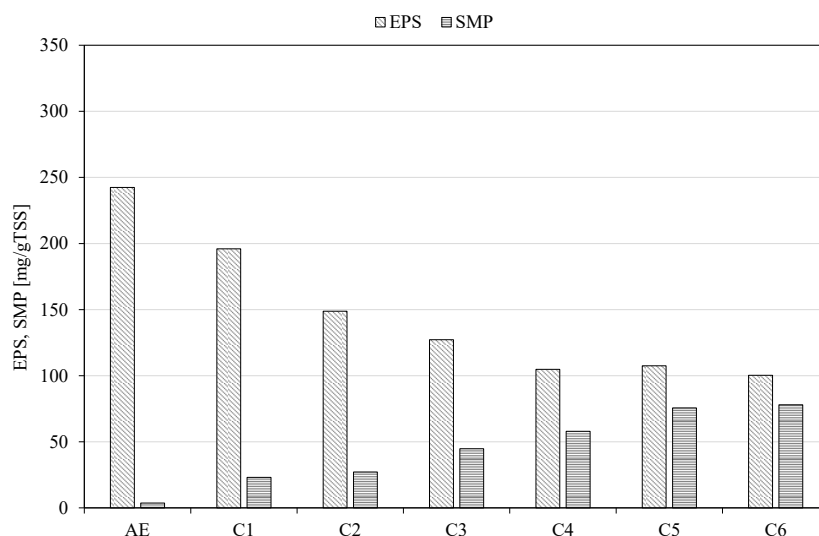


Figure 4: Concentration of the EPS and SMP in the aerobic reactor and the ASSR into all the six compartments

The EPS measured in the aerobic reactor showed the highest concentration (approximately 243 mg/gTSS), whereas SMP were almost negligible (approximately 3 mg/gTSS), indicating a floc structure mainly sustained by a dense matrix of bound polymeric substances.

When sludge was treated in the ASSR, a clear and progressive modification of the organic matrix was observed along the reactor. EPS decrease from about 196 mg/gTSS in C1 to about 148 mg/gTSS in C2 and to about 127 mg/gTSS in C3, reaching values close to 105 mg/gTSS in C4 and remaining low in C5 and C6 (approximately 108 and 100 mg/gTSS, respectively). In parallel, SMP increase steadily from about 23 mg/gTSS in C1 to about 27 mg/gTSS in C2, 45 mg/gTSS in C3 and 58 mg/gTSS in C4, attaining the highest levels in the last compartments (approximately 75 mg/gTSS in C5 and 78 mg/gTSS in C6).

The opposite trends of EPS and SMP along the six compartments provided direct evidence of a progressive deconstruction of the extracellular polymeric matrix within the anaerobic reactor. Compared with the ASSR configuration with a single compartment, the effect of EPS destruction was more marked. Indeed, results reported by Mulone et al. (2024) indicated a lower decrease in EPS concentrations from the aerobic (313 mg/gTSS) to the anaerobic reactor (272 mg/gTSS). This result suggested that the occurrence of ORP gradients within the compartmentalized ASSR promoted a greater deconstruction of the EPS matrix.

EPS constitute the structural backbone of activated sludge flocs and are mainly composed of high-molecular-weight proteins and polysaccharides (Le-Clech et al., 2006). Under anaerobic conditions, the lack of external carbon source promotes the use of these macromolecular compounds as reserve substrate. As a result, bound EPS were progressively solubilised and converted into low-molecular-weight products that accumulate in the liquid phase as SMP. The marked increase in SMP observed between C2 and C4 (from approximately 27 to 58 mg/gTSS) suggested that hydrolysis becomes increasingly relevant with increasing exposure time to anaerobic conditions. The stabilization of EPS at low levels in the final compartments indicated that most of the readily hydrolysable fraction of the polymeric matrix was already disrupted.

The progressive conversion of EPS into SMP represents a key mechanism for excess sludge reduction in the ASSR configuration. By transforming structurally bound organic matter into soluble compounds, the particulate fraction of sludge is reduced and becomes more available for further anaerobic and downstream biological conversion. This limits the accumulation of residual biomass and directly contributes to the reduction of waste activated sludge production.

From an operational and solids performance perspective, the depletion of EPS is expected to significantly affect sludge settling properties. Indeed, EPS play a central role in inter-particle bridging, floc cohesion and mechanical stability (Wanner, 2021). Their progressive removal along the compartments is therefore likely to weaken the floc structure and promote the formation of smaller and more fragile aggregates. This may result in a deterioration of settleability, due to lower floc density and reduced resistance to hydrodynamic stress, with a potential increase in dispersed solids.

Overall, the results indicated that, in the compartmentalized anaerobic side-stream reactor with six completely mixed reactors in series, EPS hydrolysis and solubilisation represented a key pathway for excess sludge reduction.

### 3.4 Plant performance

The effluent quality parameters were monitored in order to assess whether sludge reduction was achieved without impairing treatment performance. The results obtained under CAS operation (baseline condition), revealed overall high treatment efficiency, with COD removal of approximately 97% and effluent COD concentrations below 10 mg/L. Total suspended solids in the effluent was on average 16 mg/L, despite the relatively high SVI already observed (183 mL/gTSS). When the ASSR was implemented, in both the 1-C and 6-C configurations the overall effluent quality was not substantially compromised despite changes in sludge properties. Indeed, under the 1-C configuration, the effluent COD was lower than 15 mg/L at both the investigated AETs. Any significant increase in TSS concentration was observed respect to CAS operation, resulting equal to 26 mg/L and 28 mg/L at AET of 8 h/d and 10 h/d, respectively. Similarly, the settleability of the sludge was unchanged (SVI = 168 mL/gTSS). When the most compartmentalized ASSR configuration (6-C) was implemented, a moderate deterioration in effluent quality was observed, consistent with EPS destructuration and the associated worsening of sludge settleability. At the highest aeration exposure time (AET = 10 h/d), effluent COD concentrations increased to about 45 mg/L, corresponding to a removal efficiency still exceeding 93%. Similarly, effluent TSS concentrations increased, reaching average values of approximately 41 mg/L, in line with the higher SVI measured in this configuration. Nevertheless, even under the most severe operating conditions (6-C), COD removal efficiencies remained above 93%, and effluent TSS concentrations were within ranges commonly considered acceptable for secondary treatment. These results demonstrate that sludge reduction and process intensification were not associated with a substantial loss of depurative performance.

### 4. Conclusions

Results reported in this study indicated that anaerobic reactor hydrodynamics play a crucial role in excess sludge minimization in ASSR-based processes. Highly compartmentalized anaerobic reactors enhanced metabolic stress on the biomass, leading to higher sludge reduction yields, even at identical anaerobic exposure times. These findings highlight that the compartmentalisation of the ASSR is a promising design option for optimizing ASSR systems aimed at reducing excess sludge production.

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