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# Aerobic granular sludge formation in a sequencing batch reactor treating agro-industrial digestate

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**Abstract.** Most of nitrogen emissions can be ascribed to agro-industrial activities. Since digestate produced by fermentation of agro-industrial residues can be difficult to dispose of due to its high ammonium content, advanced technical- and cost-effective technologies must be developed and applied in order to significantly reduce its impact on the environment. In this study, aerobic granules were successfully cultivated in a granular sludge sequencing batch reactor (GSBR) fed with the ammonium-rich (approx. 2,500 mg $L^{-1}$) effluent of a 3-stage anaerobic digester treating agro-industrial residues. The peculiar characteristics of such wastewater required a 2-step operating strategy aimed at the selection of nitrifying biomass (Step 1) and the formation of aerobic granular sludge (Step 2). During Step 1, nitrifying biomass selection was achieved by properly regulating the cycle length: $NH_4^+$-N removal rates progressively increased from 42 to 109 $mg_N$ $L^{-1}d^{-1}$, and a corresponding increase in $NH_4^+$-N specific removal rates from 8 to 24 $mg_N$ $g_{VSS}^{-1}d^{-1}$ was also observed. During Step 2, the increase in selective pressures (i.e., minimum settling velocity and volumetric organic loading rate) led to the formation of compact (average diameter, 1.02±0.43 mm) and well settling granules ($SVI_5$, 28.6±3.8 mL $g_{TSS}^{-1}$), which were able to remove up to 89±2% of organic matter (as COD), 79±3% of $NH_4^+$-N and 59±4% of nitrogen (as sum of $NH_4^+$-N, $NO_2^-$-N and $NO_3^-$-N). The 2-step operating strategy played a key role in biomass selection and subsequent granule formation and maintenance in the GSBR, and may be successfully adopted for the treatment of different ammonium-rich wastewaters.

**Keywords**: Aerobic granules; agro-industrial wastewater; ammonium; granulation; nitrification.

## INTRODUCTION

In the European Union, up to 92% (3.60 Mt) of the total ammonia emissions in 2016 were produced by the agricultural sector [1]. Among potential nitrogen vectors, digestate from anaerobic digestion plants treating agro-industrial residues represents a significant contribution to nitrogen release into the environment: more than 12,700 anaerobic digestion plants have been reported in 2017 as operating in the agricultural sector, and agricultural feedstocks comprising livestock manure, farm residues, plant residues and energy crops are the driving force of the European biogas market with a 60-70% market share [2]. The anaerobic digestion of such organic substrates transfers nitrogen from the solid to the liquid phase, which is therefore characterized by high ammonium concentrations and represents, if not properly managed, a threat to the environment [3,4]. In facts, nitrogen abundance hinders direct digestate application to soils, together with other factors like transport requirements, water content, or presence of undesired substances and pathogens [5,6]. Moreover, digestate is considered a waste according to the EU Waste Framework Directive (2008/98/EC) [7], therefore its proper management represents a significant voice of cost for many of the existing anaerobic digestion plants [6]. Chemical-physical processes such as evaporation, air/steam stripping or membrane filtration are commonly used for the removal of nitrogen from digestate, although they are considered moderately effective and/or highly expensive [6,8].

For all the above mentioned, the development of advanced biological treatment technologies with low environmental impact is of increasing interest, as they may constitute a cost- and technical-effective alternative to conventional systems. Among such technologies, aerobic granular sludge may represent an interesting option due to their peculiar characteristics: in particular, the concentration of biomass in aerobic granular sludge reactors can be up to three times higher than in conventional activated sludge systems, thus resulting in higher treatment efficiency, smaller reactors (up to 40-80% reduction in land area requirements), and lower operational costs (20-60% reduction in energy consumption); moreover, the aerobic granular sludge technology is characterized by excellent sludge settleability (which implies lower sludge production due to high biomass retention), as well as by the coexistence of heterogeneous biomass within the layered granular structure, which allows the simultaneous removal of organic matter and nutrients [9,10].

Although the aerobic granular sludge technology has been successfully applied to the treatment of wastewaters characterized by low COD/N ratio and high N load, such as landfill leachate [11–13] or reject water [14], its applicability to the treatment of digestate characterized by high COD/N ratio and high organic and nitrogen loads has not been tested extensively yet. In facts, only few studies have been dedicated so far to the treatment of digestate from agro-industrial waste by aerobic granular sludge, although aerobic granules have been shown to cope well with high organic loads [15–17], and the long sludge age in granular sludge systems should ensure the growth of ammonium oxidizing bacteria [18]. Aerobic granular sludge have been recently applied to the treatment of diluted digestate produced by co-fermentation of corn-silage and liquid manure (3:7, v/v) [18]: the digestate was previously diluted with rainwater, and aerobic granular sludge was able to achieve an ammonium removal efficiency of 98.5±1.2 and 93.6±2.0% at a nitrogen loading rate of 1.0 and 3.4 $g_{TN}$ $L^{-1}d^{-1}$, respectively. The liquid phase of digestate from the same biogas plant was diluted with municipal wastewater (1:4, v/v) and treated by aerobic granular sludge coupled with an ultrafiltration unit as polishing step [6]: given a cycle length of 8 hours, granular sludge was able to remove 97.5±9.5% and 46.1±10.9% of influent ammonium and total nitrogen, respectively, and the ultrafiltration unit produced a permeate suitable for reuse.

To our knowledge, no information have been reported so far concerning process start-up and biomass selection strategies for the treatment of undiluted agro-industrial digestate by aerobic granular sludge. In this study, a 2-step operating strategy aimed at nitrifying biomass enrichment (Step 1) and stable nitrifying aerobic granules formation (Step 2) was applied for the treatment of undiluted digestate produced by an anaerobic digestion plant fed with agro-industrial residues. The evolution of aerobic granules physical and morphological properties was investigated, and process performances were evaluated in terms of nitrogen and organic matter removal efficiencies. The results may be useful for the start-up and enrichment of aerobic granular sludge reactors treating similar liquid residues with high COD and nitrogen concentration.



## MATERIALS AND METHODS

### Reactor set-up and operation

A glass reactor with a working volume of 2.5 L, an internal diameter of 11.3 cm and a working height/diameter ratio of 2.2 was used to carry out the experiments at controlled temperature (25 °C) and pH (7.0±0.2). Air was supplied at the bottom of the reactor, and the total airflow was kept at 10 NL $min^{-1}$ (the corresponding superficial gas velocity was 1.66 cm $s^{-1}$).

The reactor was inoculated with conventional activated sludge drawn from the municipal wastewater treatment plant of Cagliari, Italy (volatile suspended solids, VSS, 4.1 g $L^{-1}$; volatile suspended solids/total suspended solids ratio, VSS/TSS, 0.8), and initially operated as a batch selector in order to allow biomass acclimation to high $NH_4^+$-N concentration (Step 1). Batch duration was set depending on the observed $NH_4^+$-N concentration profiles, and a new batch was started only after complete nitrification was achieved. The volumetric exchange ratio (i.e. the ratio between the influent volume and the total working volume, VER) was set at 0.1.

The reactor was subsequently operated as a granular sludge sequencing batch reactor (GSBR) (Step 2). Cycle length was decreased from 24 to 6 hours, in order to increase the resulting volumetric organic loading rate (vOLR) from 1.4 to 5.5 $g_{sCOD}$ $L^{-1}d^{-1}$. The 6-hour cycle configuration consisted of: feeding, 3 min (pulse feed); aerated reaction, 342-350.5 min; settling, 10-1.5 min (corresponding to an increase in minimum settling velocity from 0.6 to 4.0 m $h^{-1}$); effluent withdrawal, 5 min. At the given VER (0.4), the resulting hydraulic retention time (HRT) was 15 h. The sludge retention time (SRT) was not controlled during the experimental activity.

**Influent composition**

The digestate produced by a three-stage anaerobic digester treating corn silage, triticale, livestock manure and other agro-industrial residues was preliminarily sieved at the plant site, and further sieved using a lab-scale vibrating sieve (mesh size, 250 μm) before being fed to the GSBR. Main chemical and physical characteristics of digestate are summarized in Table 1.

**Analytical methods**

Total solids (TS), total suspended solids (TSS), volatile suspended solids (VSS), COD and $NH_4^+$-N were determined according to Standard Methods [19]. Soluble COD was measured after filtering the samples using 0.45 μm acetate membrane filters. The concentrations of $NO_2^-$-N and $NO_3^-$-N were determined by ion-chromatography on filtered samples (0.45 μm) using a DIONEX ICS-90 chromatograph equipped with an IonPAC AS14A-5 μm column (DIONEX). Samples were taken from the influent, effluent, immediately after feeding ($t_0$) and during the reaction phase at fixed intervals. All samples were properly diluted with de-ionized water before analysis.

During Step 1, samples were taken at regular intervals during each batch, and maximum $NH_4^+$-N removal rates were calculated considering the steepest part of each $NH_4^+$-N concentration profile. Maximum specific removal rates were then calculated as the ratio between the maximum removal rate and the VSS concentration in the selector.

Nitrogen removal efficiency (NRE) was calculated considering nitrogen in the form of ammonium, nitrite and nitrate ($N_t = NH_4^+\text{-N} + NO_2^-\text{-N} + NO_3^-\text{-N}$), according to the following equation [20]:

$$NRE(\%) = 100 \cdot \frac{(N_t)_{inf} - (N_t)_{eff}}{(N_t)_{inf}} \qquad \text{(Eq. 1)}$$

Sludge volume index (SVI) was determined by reading the height of the settled bed after 5 and 30 minutes settling and calculated from the settled bed volume and the dry weight in the reactor. The ratio $SVI_5/SVI_{30}$ was also calculated, and used as indication of sludge bed compactness.

Image Analysis was performed on granules samples taken at the end of the feeding, under completely mixed conditions: each sample (60 mL) was put into a Petri dish on a dark background, and pictures were taken in b/w mode via a high resolution digital camera placed onto a horizontal holder. Image-Pro Plus v.6 was used to determine granule size (mean diameter, i.e., the average length of diameters measured at 2 degrees intervals and passing through granule centroid). More than 100 granules were considered for each sample.

## RESULTS AND DISCUSSION

### Step 1 – Preliminary acclimation

During Step 1, the reactor was operated as a batch selector, and batch duration was set depending on the observed $NH_4^+$-N concentration profiles (i.e., a new batch was started only after complete nitrification was achieved). Such dynamic operating strategy was aimed at favoring biomass acclimation to high nitrogen concentrations and increasing the activity of ammonium oxidizing bacteria, rather than promoting granules formation.

In batch #1, a significant decrease in ammonium oxidation rate was observed between days 2 and 3 (Figure 1). Such behavior was related to the automatic pH control within the range 7.0±0.2, which required significant acid dosage into the reactor in the very early stage of the batch, with the consequent complete consumption of alkalinity. In facts, since 2 moles of alkalinity are required for the conversion of 1 mol of ammonium into nitrite [8], the alkalinity to nitrogen ratio determines the extent of $NH_4^+$-N oxidation, and can be used to regulate the amount of $NH_4^+$-N to be converted into $NO_2^-$-N. As pH control was disabled and sufficient alkalinity was restored by manual dosage of sodium bicarbonate (day 3), ammonium oxidizing activity was restored and the removal of all the residual ammonium was achieved within day 6.

In the subsequent batches (#2 and #3), no automatic pH control was applied and $NH_4^+$-N removal was successfully achieved without any lag, within 3 days of operation. The pH in the selector self-regulated at around 6.5-7.0 due to acidification caused by nitrification, which showed a much higher rate compared with Batch #1: as shown in Figure 2, $NH_4^+$-N removal rates progressively increased from 42 to 109 mg $L^{-1}d^{-1}$, and a corresponding increase in $NH_4^+$-N specific removal rates from 8 to 24 mg $g_{VSS}^{-1}d^{-1}$ was also observed, indicating the successful acclimation and enrichment of nitrifying biomass.

During Step 1, no nitrite formation was observed, and $NH_4^+$-N conversion into nitrate was confirmed by the accumulation of $NO_3^-$-N into the reactor. Most of the organic matter was removed within few hours from the beginning of each batch: the observed removal efficiencies were up to 60% (as COD) and did not change significantly from batch #1 to #3.

### Step 2 – Granular sludge formation

Different hypotheses have been proposed for explaining aerobic granular biomass formation in sequencing batch reactors. Among them, the four-step hypothesis based on cell to cell interactions (1), attachment of bacteria and formation of aggregates (2), enhanced attachment by EPS production (3) and shaping of granules in aerobic granulation (4) is a widely accepted model for aerobic granular sludge formation [21]. Hydraulic shear stress, feast-famine regime, and short settling times are considered important triggering and selective forces for aerobic granular sludge formation and selection in sequencing batch reactors [22].

As significant nitrifying activity was achieved in Step 1, the reactor was operated as a GSBR and selective pressures such as vOLR and minimum settling velocity were increased (up to 5.5 $g_{sCOD}$ $L^{-1}d^{-1}$ and 4.0 m $h^{-1}$, respectively) in order to sustain granule formation.

The progressive increase in vOLR from 1.4 to 5.5 $g_{sCOD}$ $L^{-1}d^{-1}$ (days 0-32) was accompanied by the formation of very small aggregates (average mean diameter, < 200 μm), although floc-shaped biomass remained dominant in the system. As shown in Figure 3, sludge settleability and sludge bed compactness did not change significantly, as indicated by $SVI_5$ (103±5 mL $g_{TSS}^{-1}$) and $SVI_5/SVI_{30}$ (1.46±0.04) values.

Although increasing the applied vOLR triggered the formation of small aggregates, its contribution alone to aerobic granulation was poor. Such result was somehow expected, since vOLR mostly influences the physical characteristics of already formed aerobic granules, rather than allowing granules formation and selection [23]: as previously reported, the mean size of aerobic granules increased from 1.6 to 1.9 mm with the increase of vOLR from 1.5 to 9 $kg_{COD}$ $m^{-3}d^{-1}$ [24]; aerobic granules developed at different vOLRs were characterized by similar density, specific gravity and SVI, and their physical strength decreased as vOLR increased [24-27], suggesting that higher vOLRs can enhance biomass growth rate and, in turn, reduce the structural strength of the microbial community [28]; moderate vOLR was found to favor aerobic granular sludge stability [16].

The subsequent, progressive increase of minimum settling velocity from 0.6 to 4.0 m $h^{-1}$ (days 33-61) had a much more significant impact on granule formation and selection, as well as on sludge settling properties. In particular, aerobic granules progressively grew in number and size: average granule mean diameter increased up to 0.6 mm, as shown in Figure 4. Such increase in granules size was accompanied by a corresponding decrease of $SVI_5$, $SVI_{30}$ and $SVI_5/SVI_{30}$ (Figure 3), which rapidly dropped down to 50 mL $g_{TSS}^{-1}$ (68.7±19.0 mL $g_{TSS}^{-1}$), 45 mL $g_{TSS}^{-1}$ (52.5±10.8 mL $g_{TSS}^{-1}$) and 1.12 (1.29±0.10), respectively, indicating an improvement of sludge settleability.

From day 61 onward, granules average mean diameter further increased (1.02±0.43 mm), and granules settling properties stabilized, indicating that mature granule formation was successfully achieved (Figure 3): under steady state conditions (days 130-170), average $SVI_5$, $SVI_{30}$ and $SVI_5/SVI_{30}$ were 28.6±3.8 mL $g_{TSS}^{-1}$, 27.8±3.6 mL $g_{TSS}^{-1}$ and 1.03±0.02, respectively. Such results were comparable with those achieved in previous studies with simulated and real industrial wastewaters [17,29–31], landfill leachates [11,32] and different anaerobic digestates: in particular, Cydzik-Kwiatkowska et al. [14] reported $SVI_5$ values within the range of 25–30 mL $g_{TSS}^{-1}$ in a GSBR treating diluted and undiluted anaerobic digester supernatant at different cycle lengths (6, 8, 12 h); Świątczak et al. [6] reported SVI of about 50 mL $g_{MLSS}^{-1}$ in a GSBR treating the liquid phase of digestate from manure co-digestion mixed with municipal wastewater; Świątczak and Cydzik-Kwiatkowska [18] observed well settling aerobic granules characterized by low $SVI_{30}$ values (44.4±3.9 and 41.9±0.4 mL $g_{MLSS}^{-1}$) in a GSBR treating diluted digestate produced by co-fermentation of corn-silage and liquid manure, at different nitrogen loading rates (1.0 and 3.4 $g_{TN}$ $L^{-1}d^{-1}$, respectively).

In this study, increasing the minimum settling velocity played a key role in the formation and selection of granular aggregates: although formation of aerobic granules can take place when minimum settling velocity is above 1 m $h^{-1}$, aerobic granular sludge becomes dominant only when minimum settling velocities of at least 4 m $h^{-1}$ (i.e., the same used in our study) are applied [22]. For this reason, reactors are typically operated with short settling times of 2-10 min, which force the bacteria to form granular aggregates with enhanced settling properties, while floc-shaped sludge with poor settleability is washed out [21]: as reported previously, aerobic granules were successfully cultivated and became dominant only in a SBR operated at a settling time of 5 min, while mixtures of aerobic granules and suspended sludge were observed when the SBRs were run at settling times of 20, 15, and 10 min [33]. Since the formation of aerobic granular sludge is not immediate, the operating strategy adopted in our study was to progressively increase the minimum settling velocity, in order to avoid complete biomass washout.

**Step 2 - GSBR performance**

As to GSBR performance, nitrogen removal did not show any significant change as long as the selective pressure was kept low. As shown in Figure 5, the increase in minimum settling velocity up to 4.0 m $h^{-1}$ was accompanied by an increase in nitrogen removal efficiency (NRE) and ammonium removal efficiency, which averaged at 39±17% and 71±10%, respectively (days 61-170). Under steady-state conditions (days 130-170), average NRE was 59±4%, which is comparable with nitrogen removal efficiencies achieved in previous studies. Cydzik-Kwiatkowska et al. [14] observed complete ammonium oxidation in GSBRs treating diluted (approximately 240 $mg_N$ $L^{-1}$) and undiluted (474 $mg_N$ $L^{-1}$) anaerobic digester supernatant with different cycle lengths (6, 8 and 12 h); the concentrations of oxidized nitrogen forms (as $NO_2^-$-N + $NO_3^-$-N) were statistically higher in the effluent from the GSBR treating undiluted supernatant (380 vs 185 mg $L^{-1}$), as well as the time required for complete ammonium removal (up to 6, 4 and 7 h in GSBR operated at 6-, 8- and 12-hour cycle length, respectively); the NRE in the GSBRs treating diluted and undiluted supernatant was 23% and 20%, respectively; total nitrogen (as the sum of organic and inorganic nitrogen forms) was removed with an efficiency of 35% and 30%, respectively. It must be considered that substrate composition (i.e., high concentration of ammonium and slowly biodegradable organic compounds) inhibited effective granulation, which could be achieved only after the addition of acetate (the resulting COD/N ratio increased to about 2.4, and the BOD/COD ratio to 0.68). Differently from Cydzik-Kwiatkowska et al. [14], the addition of a readily degradable carbon source like acetate was not necessary in our study, likely due to the higher resulting COD/N ratio (about 3.6) and vOLR (5.5 $g_{sCOD}$ $L^{-1}d^{-1}$). Świątczak and Cydzik-Kwiatkowska [18] achieved 98.5±1.2% ammonium removal, 64.9±9.8% total nitrogen removal, and around 70% NRE in a GSBR treating diluted digestate produced by co-fermentation of agricultural waste and manure, at a nitrogen loading rate of 1.0 $g_{TN}$ $L^{-1}d^{-1}$; as the nitrogen loading rate increased to 3.4 $g_{TN}$ $L^{-1}d^{-1}$, ammonium removal, total nitrogen removal, and NRE averaged at 93.6±2.0%, 30.2±2.6%, and approximately 75%, respectively. Świątczak et al. [6] treated the liquid phase of digestate from manure co-digestion (2.8 $g_N$ $L^{-1}$) mixed with municipal wastewater (1 to 4 ratio) in an 8-hour cycle GSBR: ammonium removal, total nitrogen removal, and NRE were 97.5±9.5%, 46.1±10.9%, and 41%, respectively, and the effluent was sent to an ultrafiltration step for further treatment in view of water reuse.

In the present study, if average $NH_4^+$-N effluent concentrations are considered, ammonium levels were still high (525±73 mg $L^{-1}$), compared with previous studies concerning agro-industrial digestate treatment by aerobic granular sludge (Table 2). Such outcome is likely related to the much higher influent $NH_4^+$-N concentration in the agro-industrial digestate treated in our study, and to the relatively short cycle length applied, which did not allow complete ammonium removal. In our study, nitrate was the dominant oxidized nitrogen form in the effluent, indicating that full nitrification pathway occurred, in agreement with previous studies [18,34]. Nitrite accumulation was observed by [6,14], and related to the inhibiting effect of free ammonia (FA) on nitrite oxidizing bacteria (NOB). Although FA was reported to inhibit NOB if concentration in the bulk liquid is above the range 0.1-4 $mg_N$ $L^{-1}$ [35,36], it must be considered that actual inhibiting effect of FA on NOB has been proved to be of less importance, compared with other process parameters [37]. The highest FA concentration measured in our GSBR at the beginning of the cycle was approximately 5 $mg_N$ $L^{-1}$. However, no significant NOB inhibition was observed, likely due to the occurrence of different factors like prolonged biomass exposure to high ammonium (and FA) concentrations which may have favored NOB acclimation; the granular layered structure, which may have preserved NOB from relatively high FA concentrations in the bulk liquid due to diffusion limitation, as previously observed with other toxic and inhibiting compounds [17,29,38]; a drop of initial FA concentration due to biomass activity and air stripping, which may have reduced its inhibiting potential.

Different nitrogen removal patterns were previously described in literature, concerning aerobic granular sludge [39-41]. Based on microbial composition, heterotrophic nitrification and aerobic denitrification were suggested as the most important metabolisms involved in nitrogen removal when high carbon to nitrogen ratios are applied, with autotrophic nitrifiers and heterotrophic anoxic denitrifiers playing a supporting role: *Thauera* sp. are capable of heterotrophic nitrification and aerobic denitrification, and they were dominant in granules drawn from GSBR treating agro-industrial diluted digestates [18]. An important advantage of heterotrophic nitrification and aerobic denitrification is that such processes take place simultaneously at high organic loadings, and the simultaneous nitrification/denitrification avoids acidification in the reactor [40, 41].

In our study, the highest NRE (59±4%) was observed when mature granules with bigger average mean diameter were retained in the GSBR (days 130-170): this may be related to the consequent increasing thickness of the anoxic zone within granular layered structure due to limited oxygen diffusion, thus suggesting that denitrification was mostly carried out by heterotrophic anoxic denitrifiers, rather than aerobic denitrifiers. Indeed, the presence of different redox conditions within granules allows aerobic nitrification and anoxic denitrification to occur simultaneously when aeration is provided. Main parameters influencing simultaneous nitrification and denitrification (SND) are dissolved oxygen concentration in the bulk liquid, size of granules, electron donor availability and microbial activity [22]. Given the right electron donor availability and sufficient microbial activity, SND is not efficient if granule size and dissolved oxygen concentration are not well balanced: in particular, nitrification will be favored and denitrification will be hindered if granule size is too small and dissolved oxygen concentration is too high, and *vice versa*. Li and Liu [42] calculated the parameters of substrate and oxygen diffusion in the granules: at acetate and oxygen concentrations of 400 $mg_{COD}$ $L^{-1}$ and 8 mg $L^{-1}$, respectively, dissolved oxygen was the main limiting factor when the size of granules exceeded 0.5 mm. Despite the intense aeration provided in our study, denitrification was not hindered because oxygen was depleted in the outer granules layer, as previously reported also by Świątczak et al. [6] who achieved successful SND in smaller aerobic granules (only 12.7 % of total granules had a diameter > 1mm) cultivated in a GSBR treating diluted digestate at high organic and nitrogen loading rates, and operated with intense aeration.

Differently from nitrogen, organic matter removal efficiency showed an increasing trend already at the early stage of Step 2 (days 0-33), when the vOLR was increased from 1.4 to 5.5 $g_{sCOD}\ L^{-1}d^{-1}$ (Figure 6), in agreement with previous studies concerning digestate treatment by aerobic granular sludge: a significant increase in COD removal efficiency from 33.7±14.2% to 45.2±2.2% was observed by Świątczak and Cydzik-Kwiatkowska [18] in a GSBR treating diluted digestate as the influent COD concentration was increased from 2.4±0.1 to 9.5±0.1 g $L^{-1}$.

In the present study, the further increase in COD removal efficiency up to 89±2% (days 130-170) correlated well with the increase in aerobic granules average mean diameter: such behavior can be likely ascribed to the combined effect of thicker anoxic/anaerobic zones inside granules and the high organic load applied, which enhanced heterotrophic denitrification inside the granules. As a confirmation, a positive correlation between nitrogen removal via SND and granules size was also observed in GSBRs treating synthetic wastewater [43].

**Practical considerations**

Under steady-state conditions (days 130-170), aerobic granular sludge showed high removal efficiencies in terms of COD and $NH_4^+$-N (approximately 90% and 80%, respectively), and good removal efficiencies in terms of nitrogen (approximately 60%). Despite such good process performance, GSBR effluent did not meet quality standards for direct discharge into water bodies, nor for water reuse, due to the very high influent concentrations.

A similar outcome was observed in previous studies concerning the treatment of agro-industrial diluted digestate and diluted/undiluted digestate supernatant by aerobic granular sludge [6,14,18,34]. Treatment sequences consisting of aerobic granular sludge followed by different post-treatment steps were tested or postulated, in order to achieve high quality final effluents: the possibility to apply post-denitrification and total suspended solids separation (e.g., by ultrafiltration) to the effluent of a GSBR treating diluted agro-industrial digestate was proposed for complete nitrogen removal [18]; membrane filtration (namely ultrafiltration) was applied to the effluent of a GSBR treating diluted digestate supernatant in order to significantly reduce ammonia and organic nitrogen, suspended solids, COD and color, and possible reuse of $NO_x$-N rich permeate was suggested, in line with a circular economy based approach [6]; the effluent of a GSBR treating the diluted liquid phase of digestate from an agricultural biogas plant was sent to post-denitrification and ultrafiltration steps, thus allowing the significant reduction of high residual nitrate, COD, and total suspended solids concentrations, in the perspective of subsequent water reuse [34]; post-denitrification and ultrafiltration were applied to the nitrogen and organics rich effluent of a GSBR treating undiluted agro-industrial digestate supernatant, and a coagulation/sedimentation step prior to ultrafiltration was suggested to further increase the efficiency of organics removal [14]. Table 2 sums up the applied/postulated post treatment solutions.

Based on GSBR effluent characteristics observed in our study, different possible treatment sequences can be suggested: aerobic granular sludge can be followed by one-step partial nitritation/anammox (anaerobic ammonium oxidation) and ultrafiltration, in order to remove ammonium nitrogen and organic compounds significantly. In this case, the amount of readily degradable COD should be low enough to avoid any competition among anammox bacteria and heterotrophic denitrifiers. Alternatively, the effluent from the GSBR can be sent to a microbial electrolysis cell for the simultaneous removal of readily degradable COD and the recovery of ammonium (as ammonium sulphate) [44], and then to an ultrafiltration step. In both cases, the final effluent would still contain high concentrations of nitrates, which can be used as a source of nutrients within a biorefinery concept: for instance, microalgae cultivation for energetic purposes is characterized by high costs mostly related to nutrient supply, which hinders its commercial application [45]. Since $NO_x$ concentration in water used for algae cultivation is within the range 16-800 mg $L^{-1}$ [46,47], nitrate rich effluent may be used without dilution as a low-cost source of nutrients, as previously suggested also by [6,34].

## CONCLUSIONS

The possible application of aerobic granular sludge to the treatment of agro-industrial digestate characterized by high COD/N ratio, organic matter and nitrogen concentrations was investigated. To promote biomass acclimation to high ammonium concentration and sustain granule formation and retention in the GSBR, a 2-step operating strategy based on batch selector (Step 1) and progressively increasing selective pressure (Step 2) was applied. Successful biomass acclimation was achieved, and $NH_4^+$-N specific removal rates increased from 8 to 24 mg $g_{VSS}^{-1}d^{-1}$. The increase in vOLR did not lead to any significant effect on granule formation, while the progressive increase in minimum settling velocity led to the quick formation of compact, well settling granules which showed good NRE (59±4%), as well as high ammonium (79±3%) and organic matter (89±2%) removal capabilities under steady state conditions (days 130-170). Despite such good process performance, a treatment sequence should be adopted in order to achieve an effluent suitable for direct discharge in water bodies, or even for water reuse. Results are promising, and provide useful information for the treatment of other similar agro-industrial residues by aerobic granular sludge.

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### Declaration of interest

The Authors declare no conflict of interest

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**Figure 2.** Evolution of $NH_4^+$-N removal rates and specific removal rates observed during Step 1.

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**Table 1.** Main chemical and physical characteristics of liquid fraction of digestate collected at plant site

| Parameter | u.m. | Value |
|---|---|---|
| $NH_4^+$-N | mg $L^{-1}$ | 2,470±105 |
| COD | mg $L^{-1}$ | up to 9,000 |
| Soluble COD (sCOD) | mg $L^{-1}$ | 3,445±81 |
| sCOD/$NH_4^+$-N | - | 1.4 |
| Alkalinity | $g_{CaCO3}$ $L^{-1}$ | > 10 |
| Total Solids (TS) | % (w $w^{-1}$) | 1.42 |
| pH | - | > 8.0 |

**Table 2.** Characteristics of GSBR-treated agro-industrial digestates, and proposed post-treatment strategies. Influent $NH_4^+$-N concentrations are reported in brackets.

| Study | Digestate | $NH_4^+$-N (mg $L^{-1}$) | $NO_2^-$-N (mg $L^{-1}$) | $NO_3^-$-N (mg $L^{-1}$) | COD (mg $L^{-1}$) | Treatment sequence |
|---|---|---|---|---|---|---|
| This study | Liquid phase, undiuted | 525±73 (2,470±105) | 21±3 | 479±60 | Up to 1,200 | AGS+PN/AMX+UF* AGS+MEC+UF* |
| [6] | Liquid phase, diluted | 1.2±0.5 (497±42) | 263±80 | 28±8 | 1,117±128 | AGS+UF |
| [14] | Liquid phase, undiluted | Not detected (474) | 350-380 | 17.5-20 | 350-450 | AGS+PD+C/F+UF |
| [18] | Raw, diluted | 5.6±3.8 (392±91) | 0.8±0.8 | 119±6 | 1,600±300 | AGS+PD+UF* |
| [18] | Raw, diluted | 56±15 (893±116) | 116±5 | 54±9 | 5,200±300 | AGS+PD+UF* |
| [34] | Liquid phase, diluted | 8.7±7.3 (560) | 51±47 | 280±66 | 1,748±247 | AGS+PD+UF |

* the treatment sequence is only suggested;

Abbreviatons: AGS, aerobic granular sludge; PD, post-denitrification; UF, ultrafiltration; C/F, coagulation/flocculation; PN/AMX, partial nitritation/anammox; MEC, microbial electrolysis cell.

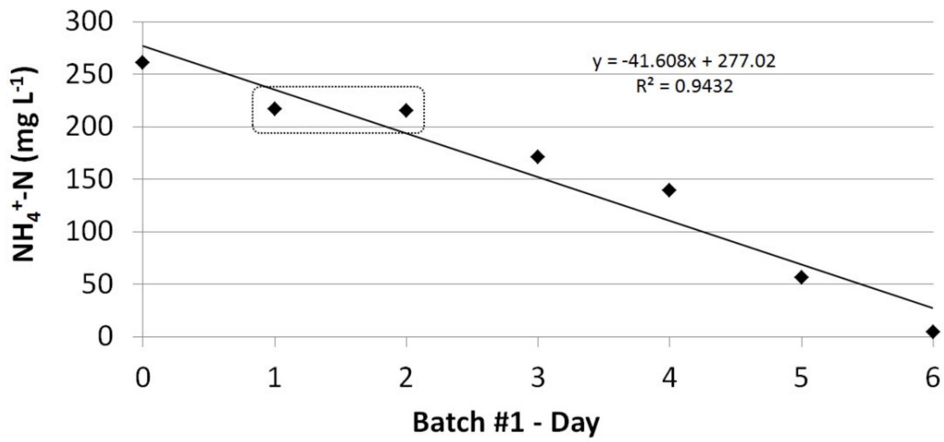


Figure 1. $NH_4^+$-N concentration profile observed during batch #1.

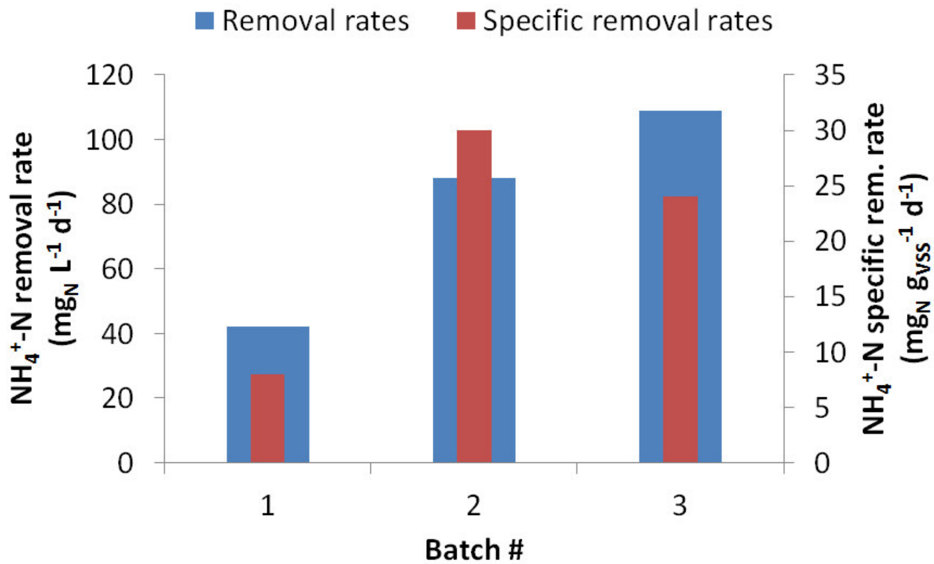


Figure 2. Evolution of $NH_4^+$-N removal rates and specific removal rates observed during Step 1.

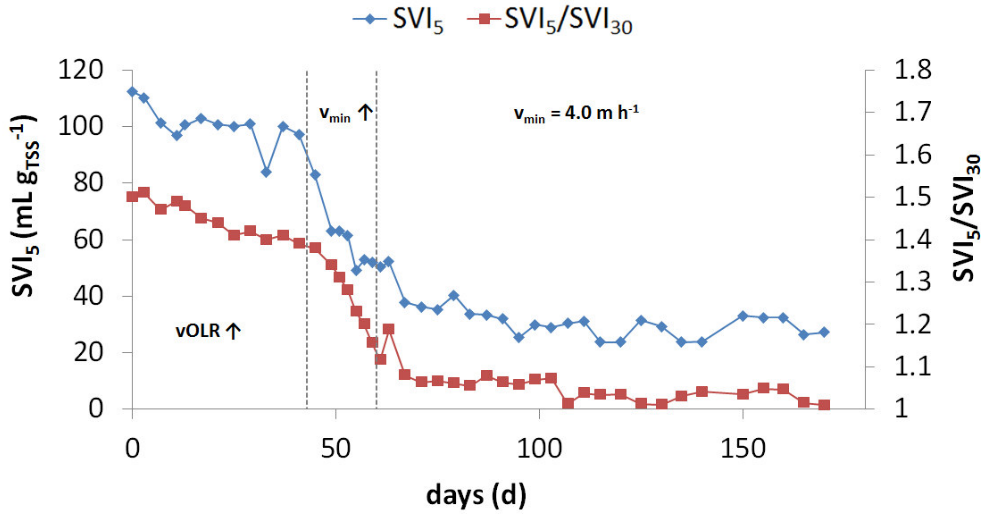


Figure 3. Sludge settling properties with different selective pressures.

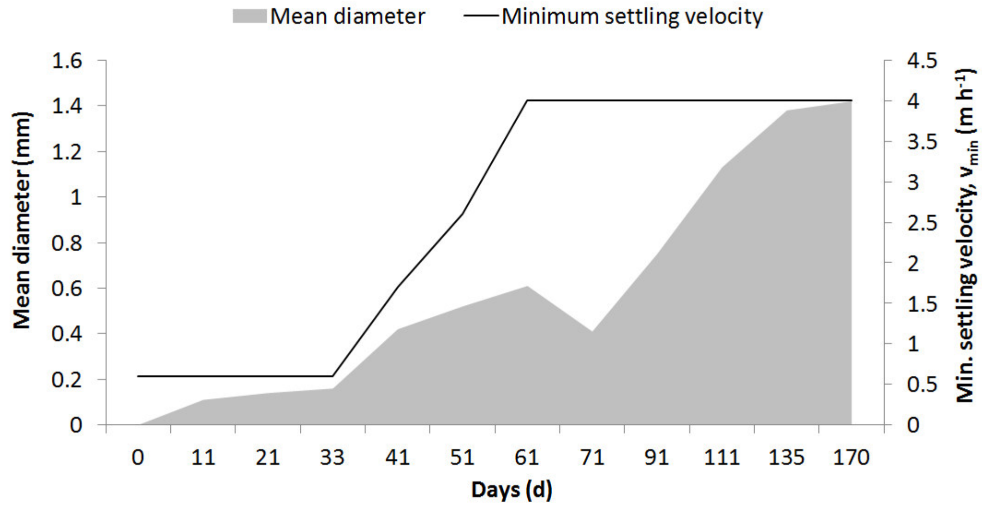


Figure 4. Evolution of granules average mean diameter during Step 2, with different minimum settling velocities.

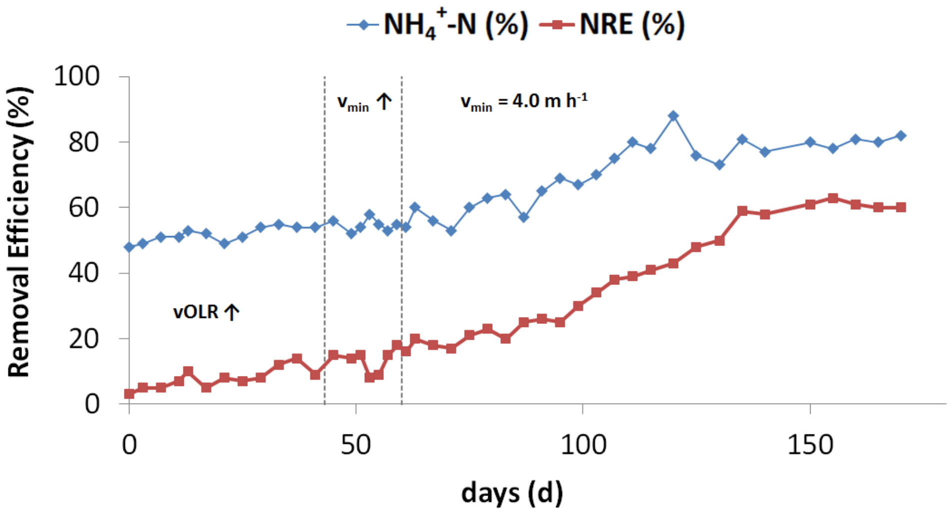


Figure 5. Nitrogen removal efficiency  (NRE) and ammonium removal efficiency observed during Step 2.

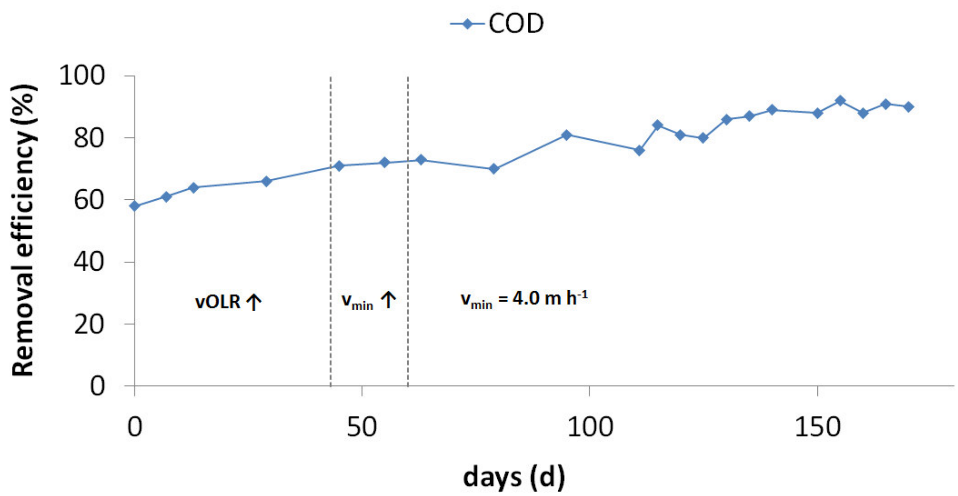


Figure 6. Organic matter (COD) removal efficiency observed during Step 2.