



Granular biomass floatation: A simple kinetic/stoichiometric explanation



J.L. Campos^{a,*}, A. Val del Río^b, A. Pedrouso^b, P. Raux^a, E.A. Giustinianovich^c, A. Mosquera-Corral^b

^a Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez, Avda. Padre Hurtado 750, Viña del Mar, Chile

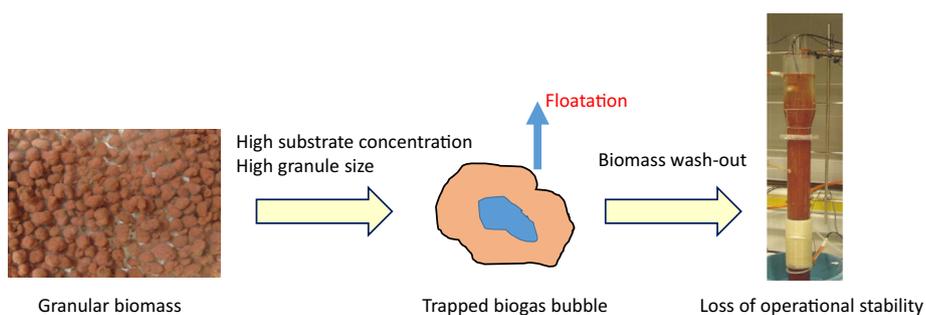
^b Department of Chemical Engineering, Institute of Technology, University of Santiago de Compostela, E-15705 Santiago de Compostela, Spain

^c Chemical Engineering Department, University of Concepción, Casilla 160-C, Concepción, Chile

HIGHLIGHTS

- Reasons of anammox, denitrifying and anaerobic granules floatation are examined.
- Substrates concentration and granules size are the main parameters to be controlled.
- Data of literature fit into the zones with risk of floatation defined in this work.
- Operating temperature could be used to control floatation episodes.

GRAPHICAL ABSTRACT



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ABSTRACT

Floatation events are commonly observed in anammox, denitrifying and anaerobic granular systems mostly subjected to overloading conditions. Although several operational strategies have been proposed to avoid floatation of granular biomass, until now, there is no consensus about the conditions responsible for this phenomenon. In the present study, a simple explanation based on kinetic and stoichiometric principles defining the aforementioned processes is provided. The operational zones corresponding to evaluated parameters where risk of floatation exists are defined as a function of substrate concentration in the bulk liquid and the radius of the granule. Moreover, the possible control of biomass floatation by changing the operating temperature was analyzed. Defined operational zones and profiles fit data reported in literature for granular biomass floatation events. From the study the most influencing parameter on floatation occurrence has been identified as the substrate concentration in the bulk media.

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1. Introduction

Biomass concentration is known to be the main factor limiting the treating capacity of biological systems. Therefore, extensive effort has been made on developing technologies with good biomass retention capacity to accumulate large biomass amounts inside the reactors. Among these technologies, those based on gran-

ular biomass are widely used. Advantages come from the fact that the granulation process is relatively easily achieved by controlling the operational conditions inside the reactor and that the investment costs are very low compared to alternatives such as the use of membranes or carrier materials [1–3]. Granulation phenomenon was firstly observed in anaerobic digesters and it was further implemented through the Upflow Anaerobic Sludge Bed (UASB) reactor concept [1], which allowed spreading the anaerobic treatment of industrial effluents. In 1997, granular biomass obtained in aerobic conditions was reported for the first time and, nowadays,

* Corresponding author.

E-mail address: j Luis.campos@uai.cl (J.L. Campos).

the developed technology is applied at full scale successfully [4]. Furthermore granular biomass is also used in several existing technologies based on the anammox process and applied nowadays at full scale [5]. Nevertheless, in the case of denitrifying systems, there are not reported applications at full scale although the formation of granular biomass is also feasible [6,7].

Taking into account that reached biomass concentrations are larger in systems with granular than suspended sludge, the former are able to treat higher loading rates. In these systems if overload conditions are imposed bubbles of produced gas (nitrogen and methane), which remain trapped inside the granules, can cause their floatation and the consequent biomass wash-out. This behavior is even more detrimental if the process taking place is inhibited by substrate which causes a loss of removal efficiency. Then, the accumulation of substrate increases causing a snowballing effect until the system totally loses its efficiency [8]. According to the observations of different authors, floatation happens when the volume of gas bubbles represents the 6–11% of the total granule volume [9–11]. Moreover, due to the low diameter of the pores inside the granules the compression pressure, caused by a biogas bubble, has been found to possibly deform and even break apart the granule [9,12].

Floatation of granules was observed to occur in laboratory scale reactors where either anaerobic, denitrifying or anammox processes took place [13–15]. However, until now the key factors causing floatation of granular sludge remain unclear since most of the research works are focused on proposing methods to avoid floatation instead of defining those operational conditions which provoke it.

Several strategies have been proposed to avoid this phenomenon. Some of them are: a) to reduce the granules size [9,16,17]; b) to increase the shear stress conditions [11,18]; c) to increase the effluent recycling ratio [19,20]; d) to increase the granules density by promoting the formation of inner precipitates [16,17,21,22] to a certain extent because an excess of precipitates formation could also decrease the biomass activity [9,23]; e) to add nutrients like phosphate [13]; f) and to decrease biomass activity by decreasing operational temperature [17]. To sum up, there is not a well-defined strategy to mitigate floatation episodes.

Furthermore, extensive research has been focused on finding the causes for floatation. In the literature some hypotheses have been provided in order to explain granules floatation such as: a) change of bacteria populations distribution throughout the granule [19]; b) change of bacteria populations [20]; c) excess growth of filamentous bacteria [24]; d) or obstruction of gas channels inside the granules by exopolymeric substances (EPS) secreted by bacteria and accumulation of biogas [9].

Again a conclusive reason is not available and for this cause, in the present study, floatation of granular biomass is explained based on kinetic/stoichiometry of the involved processes. From this analysis appropriated ranges for the values of parameters like substrate concentration and granule diameter, which avoid floatation, together with the temperature effects are supplied.

2. Materials and methods

2.1. Stoichiometric and kinetic calculations

To perform the study some initial assumptions have been made. The granules are considered spheres. The biological reactors, which contain the studied granular biomass, are considered that operate at complete mixture conditions. The external mass transfer resistance was considered negligible and, therefore, the substrate concentration on the granule surface corresponds to that present in the bulk liquid. From a physical point of view, gas pockets are formed inside granules when the concentration of a gaseous

compound in the liquid phase exceeds its solubility at the temperature of operation. Since denitrifying, anammox and methanogenic systems are generally operated at temperatures around 30 °C, N₂ and CH₄ concentrations inside the granules should not be higher than 16 mg N₂/L and 18 mg CH₄/L, respectively, in order to avoid their desorption.

A diffusion–reaction model was used to determine N₂ concentrations profiles inside anammox and denitrifying granules and CH₄ concentration profile inside methanogenic ones, and, therefore, to predict the possible formation of gas pockets. Each of the three processes will be studied separately in the following sections.

2.1.1. Anammox granular biomass

The Anammox process rate was defined as a zero order kinetic due to the low value of the affinity constants for the substrates ammonium and nitrite [25]. Then the penetration distance of a substrate inside a granule can be calculated by applying a mass balance to the compound by equaling the term corresponding to mass transfer to that of the biochemical reaction (Eq. (1)). Since anammox systems are generally fed with an excess of ammonium, to avoid inhibitory values of nitrite concentrations [26], the mass balance will be applied to the limiting substrate (nitrite).

$$\frac{1}{r^2} \cdot \frac{d}{dr} \left(r^2 \cdot D_{NO_2^-} \frac{dC_{NO_2^-}}{dr} \right) = r_{\max NO_2^-} \cdot \rho_{bA} \quad (1)$$

where $D_{NO_2^-}$: NO₂⁻ diffusivity (cm²/d); $C_{NO_2^-}$: nitrite concentration (mg NO₂⁻-N/L); $r_{\max NO_2^-}$: maximum NO₂⁻ consumption rate (mg NO₂⁻-N/g VSS d); ρ_{bA} : anammox biomass density (g VSS/L_{granule}).

By integrating this Eq. (1), between R_p and r_o , the radius value where nitrite is fully depleted (r_o) inside the granule can be calculated from Eq. (2):

$$r_o = R_p \cdot \left[\frac{1}{2} + \sin \left[\frac{1}{3} \arctg \left(\frac{3M_{T0}^2 - 2}{2\sqrt{3M_{T0}^2 - 1}} \right) \right] \right] \quad (2)$$

where R_p : granule radius (cm); M_{T0} : Thiele module for zero order reaction which is calculated as Eq. (3):

$$M_{T0} = \frac{R_p}{3\sqrt{2}} \sqrt{\frac{r_{\max NO_2^-} \cdot \rho_{bA}}{D_{NO_2^-} \cdot C_{NO_2^-} S}} \quad (3)$$

where $C_{NO_2^- S}$: NO₂⁻ concentration in the granule surface (mg NO₂⁻-N/L).

Once the value of r_o is known, a mass balance to the product (N₂) can be calculated (Eq. (4)):

$$\frac{1}{r^2} \cdot \frac{d}{dr} \left(r^2 \cdot D_{N_2} \frac{dC_{N_2}}{dr} \right) = -r_{\max N_2} \cdot \rho_{bA} \quad (4)$$

where D_{N_2} : N₂ diffusivity (cm²/d); C_{N_2} : nitrogen gas concentration (mg N₂/L); $r_{\max N_2}$: maximum N₂ production rate (mg N₂/g VSS d). This production rate can be related to $r_{\max NO_2^-}$ by considering the stoichiometry of the anammox process [27] and according to Eq. (5):

$$r_{\max N_2} = \frac{2.04}{1.32} \cdot r_{\max NO_2^-} \quad (5)$$

By substituting Eq. (5) in Eq. (4) and solving the resulting equation the profile of nitrogen gas concentration between the surface of the granule ($r = R_p$) and r_o can be calculated by means of Eq. (6):

$$C_{N_2} = C_{N_2 S} + \frac{1}{6} \frac{2.04}{1.32} \cdot \frac{r_{\max NO_2^-} \cdot \rho_{bA}}{D_{N_2}} \cdot (R_p^2 - r^2) + \frac{1}{3} \times \frac{2.04}{1.32} \cdot \frac{r_{\max NO_2^-} \cdot \rho_{bA}}{D_{N_2}} \cdot r_o^3 \left(\frac{1}{R_p} - \frac{1}{r} \right) \quad (6)$$

where C_{N_2S} : N_2 concentration in the granule surface (mg N_2/L). For values of r lower than r_o , C_{N_2} concentration is the same as in $r = r_o$.

2.1.2. Denitrifying granular biomass

The denitrification process can be considered as a zero order reaction too, since the values of the affinity constants for substrates (organic matter and nitrate) are normally lower than the concentrations present inside the reactors for both compounds [28]. Taking into account that the ratio between the maximum NO_3^- consumption rate ($r_{maxNO_3^-}$) (mg NO_3^- -N/g VSS d) and the maximum N_2 production rate (r_{maxN_2}) (mg N_2/g VSS d) is 1 and considering nitrate as the limiting substrate, the following Eq. (7) can be used to determine the nitrogen gas concentration profile throughout the granule:

$$C_{N_2} = C_{N_2S} + \frac{1}{6} \cdot \frac{r_{maxNO_3^-} \cdot \rho_{bD}}{D_{N_2}} \cdot (R_p^2 - r^2) + \frac{1}{3} \frac{r_{maxNO_3^-} \cdot \rho_{bD}}{D_{N_2}} \cdot r_o^3 \left(\frac{1}{R_p} - \frac{1}{r} \right) \quad (7)$$

where ρ_{bD} : denitrifying biomass density (g VSS/L_{granule}).

When the calculated value of M_{T0} (Eq. (3)) is lower than 0.58, nitrite/nitrate is not fully depleted inside the anammox/denitrifying granules [29] and N_2 concentration should be calculated considering r_o as zero in Eqs. (6) and (7).

2.1.3. Anaerobic granular biomass

In the case of anaerobic granules the methanogenic activity is the limiting step. The consumption rate of organic matter (acetate) by methanogenic biomass is considered as a first order reaction since the affinity constant for organic matter is relatively high and around 300 mg COD/L [30]. These values are similar to the concentration of organic matter present in the reactor as volatile fatty acids (VFA). Therefore, the mass balance inside a granule can be described by the following Eq. (8):

$$\frac{1}{r^2} \cdot \frac{d}{dr} \left(r^2 \cdot D_{COD} \frac{dC_{COD}}{dr} \right) = \frac{r_{maxCOD}}{K_{COD}} \cdot \rho_{bAn} \cdot C_{COD} \quad (8)$$

where D_{COD} : organic matter diffusivity (cm^2/d); C_{COD} : organic matter concentration (mg COD/L); r_{maxCOD} : maximum COD consumption rate (mg COD/g VSS d); K_{COD} : affinity constant of organic matter (mg COD/L); ρ_{bAn} : anaerobic biomass density (g VSS/L_{granule}).

By integrating this Eq. (8) the profile of the COD concentration throughout the granule can be calculated:

$$C_{COD} = C_{CODs} \cdot \frac{R_p}{r} \cdot \frac{\sinh \left(3 \cdot M_{T1} \cdot \frac{r}{R_p} \right)}{\sinh(3 \cdot M_{T1})} \quad (9)$$

where C_{CODs} : COD concentration in the granule surface (mg COD/L); M_{T1} : Thiele module for the first order reaction which is calculated as Eq. (10):

$$M_{T1} = \frac{R_p}{3} \sqrt{\frac{r_{maxCOD}}{K_{COD} \cdot D_{COD}}} \cdot \rho_{bAn} \quad (10)$$

Now a mass balance to the product of the reaction (CH_4) can be applied by means of Eq. (11):

$$\frac{1}{r^2} \cdot \frac{d}{dr} \left(r^2 \cdot D_{CH_4} \frac{dC_{CH_4}}{dr} \right) = -r_{CH_4} \cdot \rho_{bAn} \quad (11)$$

where D_{CH_4} : CH_4 diffusivity (cm^2/d); C_{CH_4} : methane concentration (mg CH_4/L); r_{CH_4} : CH_4 production rate (mg CH_4/g VSS d). This production rate is related to the consumption rate of organic matter taking into account that 1 g CH_4 contains 4 g COD:

$$r_{CH_4} = 0.25 \cdot \frac{r_{maxCOD}}{K_{COD}} \cdot C_{COD} \quad (12)$$

By combining Eqs. (8), (9), (11) and (12), the profile of methane concentration throughout the granule can be obtained from Eq. (13):

$$C_{CH_4} = C_{CH_4s} + 0.25 \cdot \frac{D_{COD}}{D_{CH_4}} C_{CODs} \left[1 - \frac{R_p}{r} \cdot \frac{\sinh \left(3 \cdot M_{T1} \cdot \frac{r}{R_p} \right)}{\sinh(3 \cdot M_{T1})} \right] \quad (13)$$

where C_{CH_4s} : CH_4 concentration at the granule surface (mg CH_4/L).

The profiles of N_2 concentration inside anammox and denitrifying granules and CH_4 concentration inside methanogenic granules, were calculated by using Eqs. (6), (7) and (13), respectively. For this purpose, N_2 and CH_4 concentrations at the granules surface were considered zero. In order to determine the effect of the substrate concentration on granules floatation, an operating temperature of 30 °C and a typical granule radius value of 0.2 cm were considered [30]. Once the minimum substrate concentration in the bulk liquid that promotes gas desorption was calculated, the effect of the particle size on the produced gases profiles was studied by fixing this obtained value of minimum substrate concentration.

2.1.4. Effect of temperature

Since the temperature of operation significantly affects both, biomass activity and substrate concentration in the bulk liquid, the effect of this variable on granules floatation was also studied. For this, the temperature of 30 °C was chosen as reference value for the study. Furthermore, the substrate concentration in the bulk liquid was selected as the minimum substrate concentration that promotes gas desorption calculated previously.

The substrate concentration in the liquid phase at a different temperature ($T = 20, 25$ and 35 °C) was calculated by applying a mass balance under steady state conditions for a completely mixed reactor. In the case of anammox and denitrification processes (zero order kinetics), this balance would be given by Eq. (14):

$$F \cdot (C_{No} - C_{N,T}) = \eta \cdot r_{maxN,T} \cdot X \cdot V \quad (14)$$

where F is the flowrate supplied to the system (L/d); C_{No} the inlet substrate concentration ($N = NO_2^-$ or NO_3^-) (mg N/L); $C_{N,T}$ the substrate concentration inside the system (mg N/L) at the new temperature; η the efficiency factor which is calculated according Eq. (15); $r_{maxN,T}$ the maximum specific consumption rate of substrate (mg N/g VSS d); X the biomass concentration in the system (g VSS/L); and V the system volume (L).

Table 1

Values of kinetic parameters, diffusional coefficients and biomass density used to carry out calculations.

Kinetic parameters	
r_{maxNO_2} (mg NO_2^- -N/g VSS d) (30 °C)	1585 [27]
r_{maxNO_3} (mg NO_3^- -N/g VSS d) (30 °C)	12829 [28]
r_{maxCH_4} (mg CH_4/g VSS d) (30 °C)	7100 [30]
K_{COD} (mg COD/L) (30 °C)	300 [30]
Anammox activation energy (kJ/mol)	63 [27]
Denitrification activation energy (kJ/mol)	53 [31]
Methanogenic activation energy (kJ/mol)	67 [30]
K_{COD} activation energy (kJ/mol)	-132 [30]
Diffusional coefficients	
$D_{NO_2^-}$ (cm^2/d)	1.4 [32]
$D_{NO_3^-}$ (cm^2/d)	1.4 [32]
D_{N_2} (cm^2/d)	2.2 [32]
D_{COD} (cm^2/d)	1.0 [33]
D_{CH_4} (cm^2/d)	1.7 [34]
Physical properties	
ρ_{bA} (g VSS/L)	60 [35]
ρ_{bD} (g VSS/L)	20 [36]
ρ_{bAn} (g VSS/L)	40 [37]

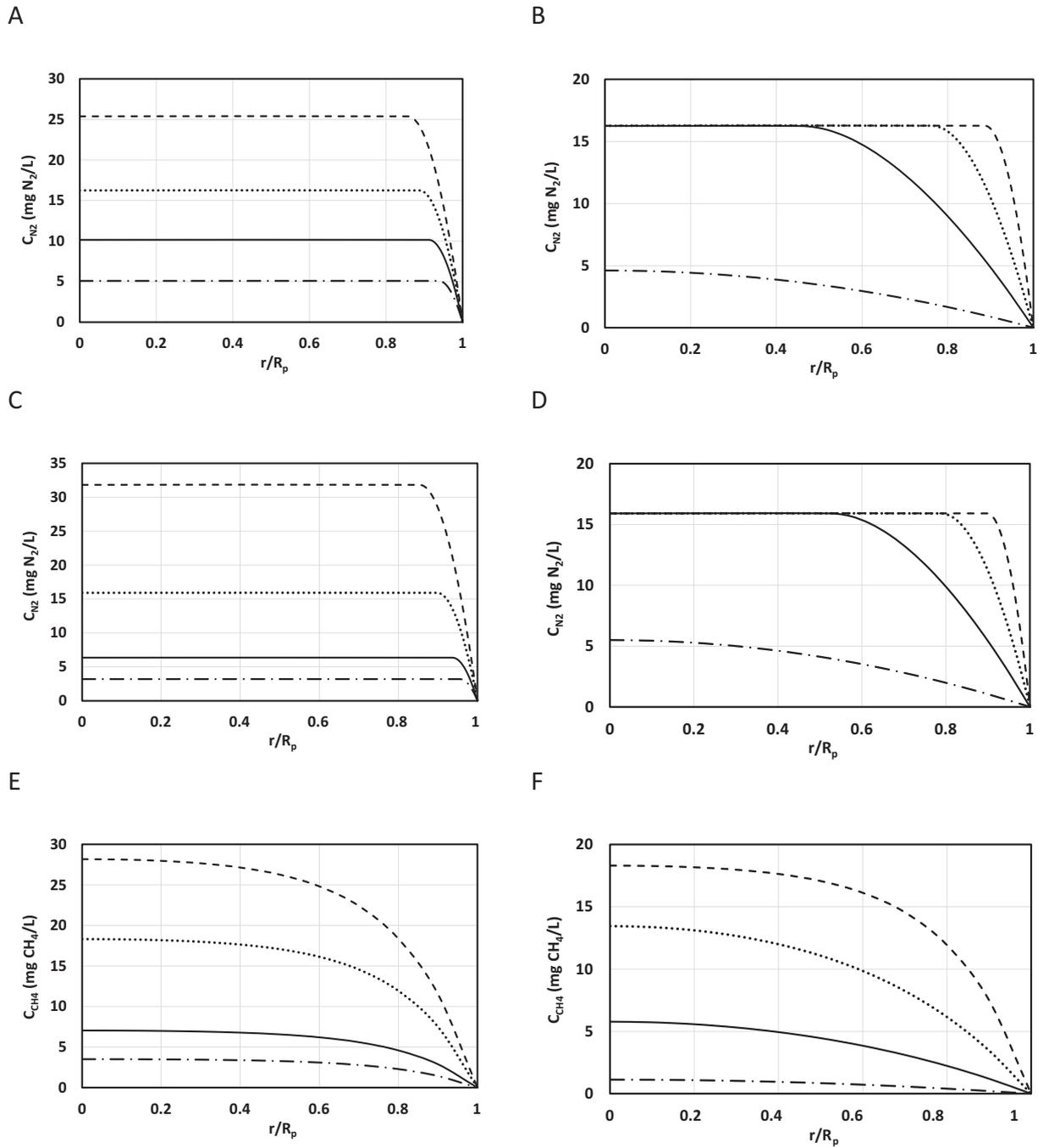


Fig. 1. Effect of substrate concentration and granule size on the profiles of N_2 and CH_4 concentrations inside Anammox, denitrifying and methanogenic granules. A) Anammox granule with a radius of 0.2 cm: Effect of substrate concentration (--- 5 mg NO_2^- -N/L – 10 mg NO_2^- -N/L 16 mg NO_2^- -N/L — 25 mg NO_2^- -N/L); B) Anammox granule at a substrate concentration of 16 mg NO_2^- -N/L: Effect of radius size (--- 0.02 cm – 0.05 cm 0.1 cm — 0.2 cm); C) Denitrifying granule with a radius of 0.2 cm: Effect of substrate concentration (--- 5 mg NO_3^- -N/L – 10 mg NO_3^- -N/L 25 mg NO_3^- -N/L — 50 mg NO_3^- -N/L); D) Denitrifying granule at a substrate concentration of 25 mg $N-NO_3^-$ /L: Effect of radius size (--- 0.02 cm – 0.05 cm 0.1 cm — 0.2 cm); E) Methanogenic granule with a radius of 0.2 cm: Effect of substrate concentration (--- 25 mg COD/L – 50 mg COD/L 130 mg COD/L — 200 mg COD/L); F) Methanogenic granule at a substrate concentration of 130 mg COD/L: Effect of radius size (--- 0.02 cm – 0.05 cm 0.1 cm — 0.2 cm).

$$\eta = 1 - \left(\frac{r_o}{R_p}\right)^3 \quad (15)$$

By dividing mass balances at 30 °C and at a generic temperature T Eq. (16) is obtained. From this equation, the substrate concentration in the liquid phase ($C_{N,T}$) is calculated, considering a value of 1000 mg/L for C_{N_0} and using Solver (Microsoft Excel).

$$\frac{(C_{N_0} - C_{N,30})}{(C_{N_0} - C_{N,T})} = \frac{\eta_{30} \cdot r_{\max N,30}}{\eta_T \cdot r_{\max N,T}} \quad (16)$$

In the case of the methanogenic process, the calculations were carried out in a similar way but considering the process as a first order kinetic. The following Eq. (17) was used to calculate the substrate concentration in the bulk liquid at different temperatures:

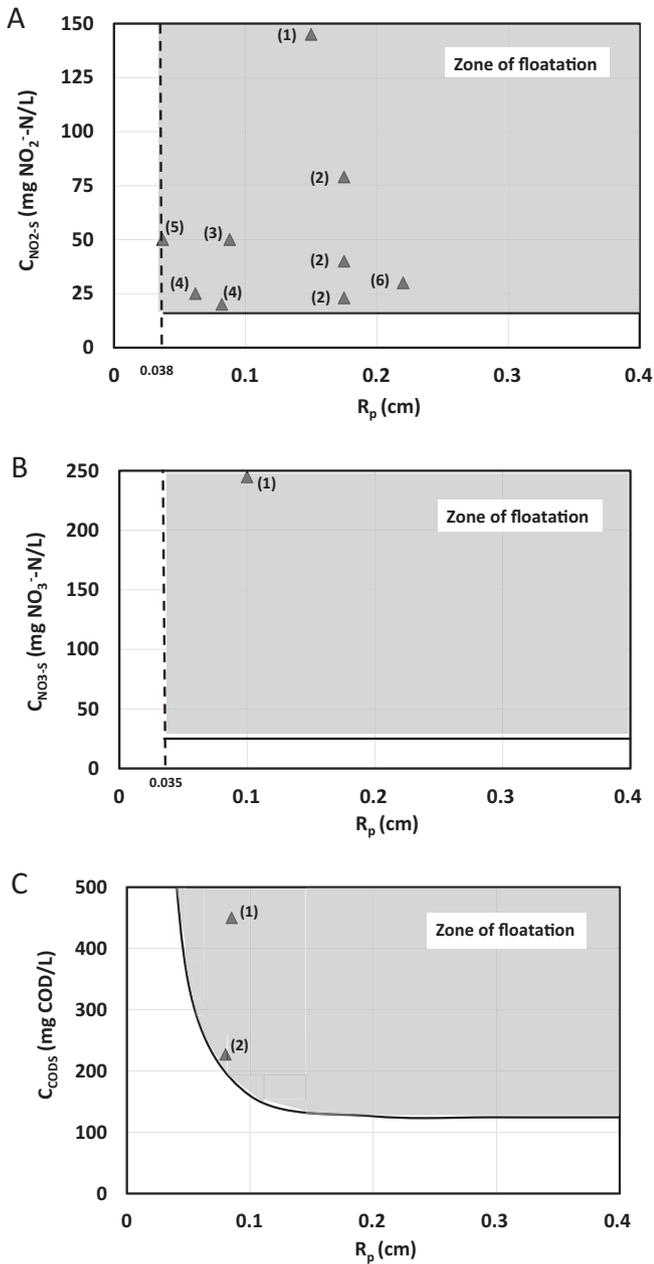


Fig. 2. Risk zone of floatation in grey for: A) anammox granules: (1) Chen et al. [9], (2) Chen et al. [39], Xing et al. [42], (4) Dapena-Mora et al. [15], (5) Ma et al. [18], (6) Yang et al. [43]; B) denitrifying granules: (1) Lee et al. [20]; and C) methanogenic granules: (1) Shin et al. [44], (2) Han et al. [45].

$$\frac{(C_{COD_0} - C_{COD,30})}{(C_{COD_0} - C_{COD,T})} = \frac{\eta_{30} \cdot \frac{r_{\max COD,30}}{K_{COD,30}} \cdot C_{COD,30}}{\eta_T \cdot \frac{r_{\max COD,T}}{K_{COD,T}} \cdot C_{COD,T}} \quad (17)$$

where C_{COD_0} the inlet substrate concentration (mg COD/L); $C_{COD,T}$ the substrate concentration inside the system (mg COD/L) at the new temperature; η the efficiency factor which is calculated according Eq. (18); $r_{\max COD,T}$ the maximum specific consumption rate of substrate (mg COD/g VSS d); $K_{COD,T}$ the affinity constant of organic matter (mg COD/L).

$$\eta = \frac{1}{M_{T1}} \left(\frac{1}{tgh(3 \cdot M_{T1})} - \frac{1}{3 \cdot M_{T1}} \right) \quad (18)$$

The values of $r_{\max N,T}$, $r_{\max COD,T}$ and $K_{COD,T}$ at 20, 25 and 35 °C were calculated from the value at 30 °C and by applying Arrhenius equation. The used values for the activation energy are detailed in Table 1. The effect of temperature on the diffusion coefficients was considered negligible.

With the values of substrate concentration in the liquid phase ($C_{N,T}$ and $C_{COD,T}$), $r_{\max COD,T}$ and $K_{COD,T}$ for each temperature the concentration profiles for N_2 and CH_4 were calculated within the granules by using Eqs. (6), (7) and (13), as it was previously described. The effect of temperature was tested for granules with radius of 0.2, 0.1 and 0.04 cm. The possible effects of substrates inhibition were not considered for results discussion.

3. Results and discussion

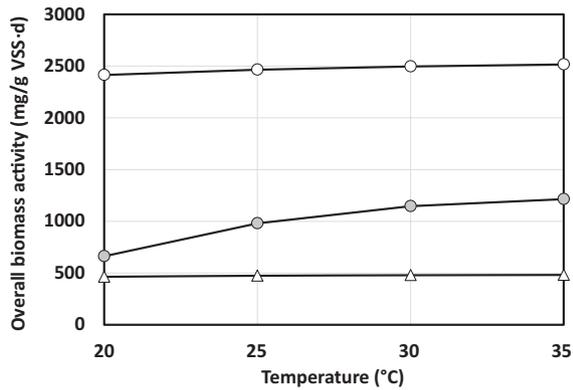
3.1. Effect of substrate concentration in the bulk liquid and granule size on granules floatation

Initially, the minimum substrate concentration value exceeding the gas solubility at 30 °C was obtained from the profiles of gas production inside the granules (Fig. 1A, C and E). For this purpose, previously described calculations were performed for substrate concentrations in the following ranges: 5–25 mg $NO_2^- - N/L$ for anammox granules, 5–50 mg $NO_3^- - N/L$ for denitrifying granules and 25–200 mg COD/L for methanogenic granules. Results indicated that gas desorption will occur inside an anammox, denitrifying or methanogenic granule with an average radius value of 0.2 cm, if substrates concentrations in the bulk liquid exceed 16 mg $NO_2^- - N/L$, 25 mg $NO_3^- - N/L$ or 130 mg COD/L (as acetate), respectively. In these cases the concentrations of nitrogen and methane present inside the granule will be higher than the limit values of 16 mg N_2/L and 18 mg CH_4/L , respectively (Fig. 1A, C and E).

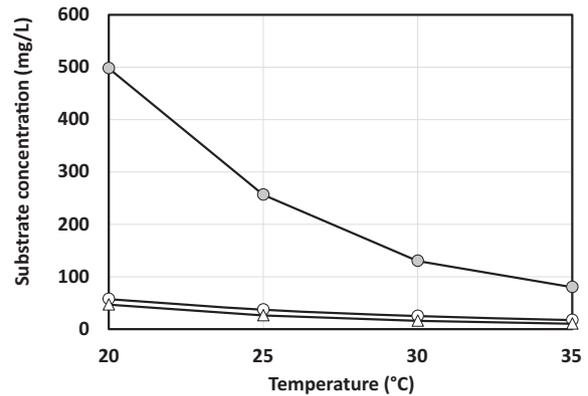
In fact, from experimental results the floatation episodes have been found to be closely related to the accumulation of substrates in the bulk liquid. Events of substrate accumulation are observed in those cases when the applied specific loading rate exceeded the maximum specific activity of the biomass [15]. In the case of the methanogenic granular biomass, these calculations are applicable only to acetate used as substrate. If other organic substrates are fed to the system, the rates of the different steps comprising the anaerobic digestion process should be taken into account and the calculation would become more complex. For these cases the concentration of total VFAs causing floatation would presumably be higher than that estimated of 130 mg COD/L [38].

Calculations to evaluate the effect of the granules size were carried out at substrate concentrations of 16 mg $NO_2^- - N/L$, 25 mg $NO_3^- - N/L$ and 130 mg COD/L (as acetate) for anammox, denitrifying and methanogenic granules, respectively. Calculations were performed for granules sizes from 0.02 to 0.2 cm. Results indicated that if the substrate concentration in the bulk liquid is enough to cause gas desorption inside the granule, the fraction of granule where gas is desorbed increases as the granule size increases (Fig. 1B and D). It implies that large granules are more prone to float than small ones. Lu et al. [10] proposed that in the case of big size granules, the inner gas has to travel a longer distance to reach the external surface than in the case of small ones. Furthermore this behavior created a larger resistance and a higher pressure of nitrogen gas inside the granules. Simultaneously higher gas accumulation inside the granule provokes its density decrease to values lower than those of liquid media (water), leading again to their floatation. This can be the reason why some authors propose the control of the granules size as a measure to avoid their floatation [9,16,17].

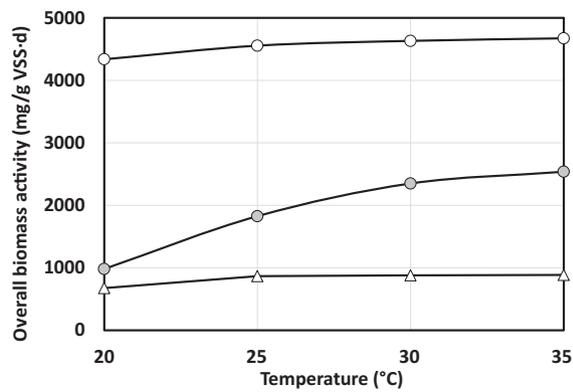
A.1



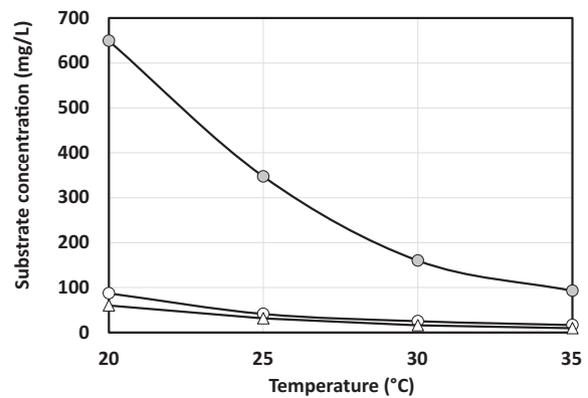
A.2



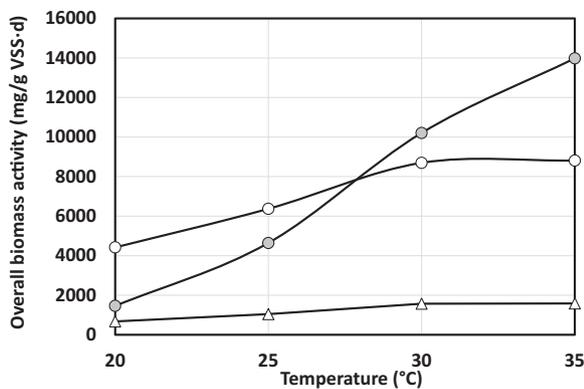
B.1



B.2



C.1



C.2

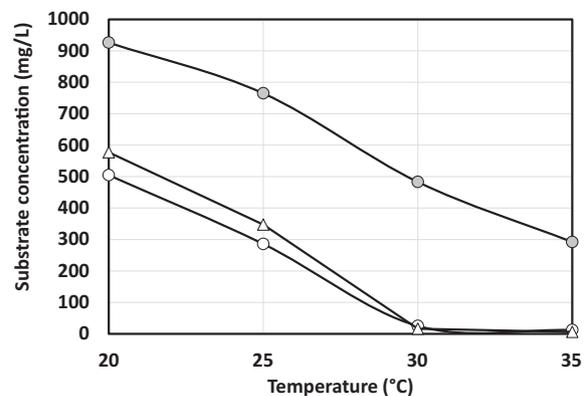


Fig. 3. Overall biomass activity and substrate concentration in the bulk liquid at different temperatures for anammox (Δ), denitrifying (\circ) and methanogenic (\bullet) granules. A) Granule with a radius of 0.2 cm; B) Granule with a radius of 0.1 cm; C) Granule with a radius of 0.04 cm. Substrates concentrations are expressed as mg N-NO₂/L (Δ), N-NO₃/L (\circ) and mg COD/L (\bullet) for anammox, denitrifying and methanogenic processes, respectively.

However, to control the size of the granules is not an easy task. On the daily basis operation of these systems dynamic events of granules growth and breakage commonly take place. Nevertheless, once granules break-up a decrease of the overall substrate consumption inside each granule is expected and as a consequence

the concentration of biogas (N₂ or CH₄) decreases, avoiding exceeding its solubility value (Fig. 1B, D and F). An effect which is not considered in these calculations is that the granules break-up, maintaining the amount of biomass in the system constant, leads to an increase of the surface of the biomass available for substrate

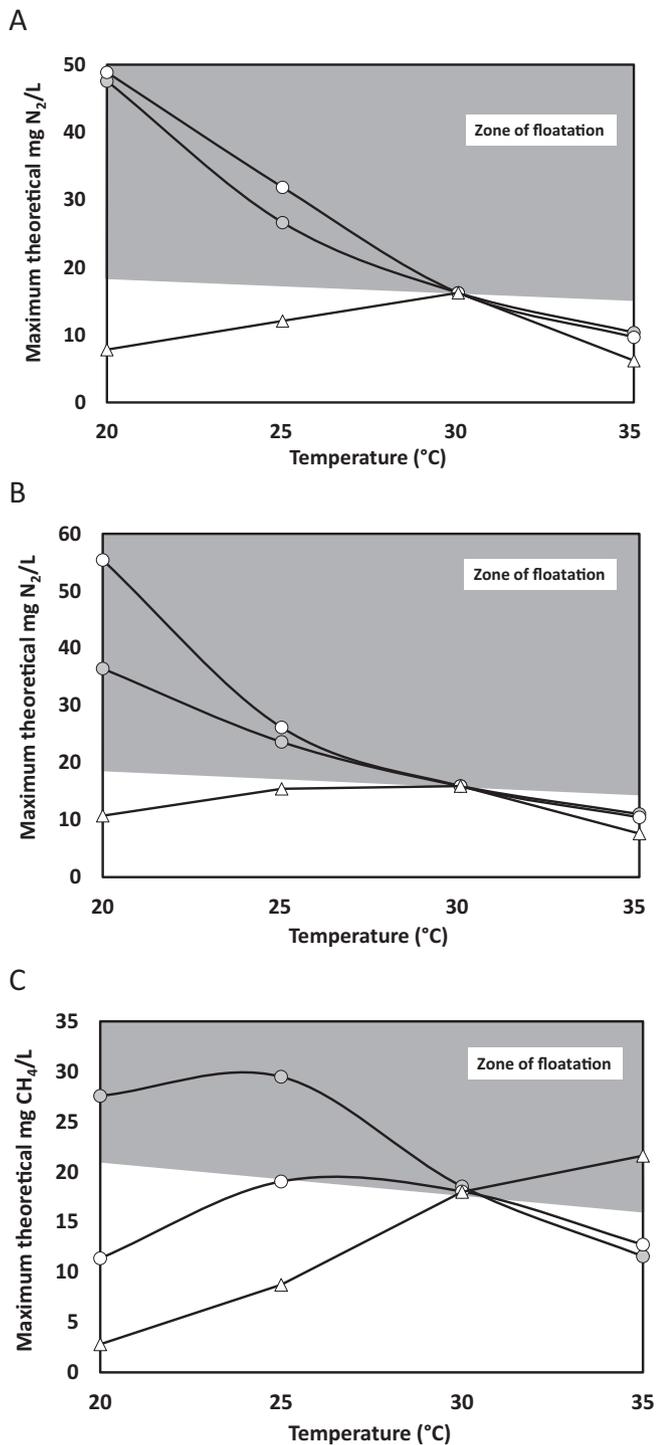


Fig. 4. Maximum theoretical gaseous products concentrations inside the granules at different temperatures for A) Anammox, B) denitrifying and C) methanogenic granules. Granule radius: 0.2 cm (●), 0.1 cm (○) and 0.04 cm (△).

transport in such a way that its consumption is promoted and therefore, its concentration in the bulk liquid decreases to safety values.

Other strategies to avoid floatation from the literature such as increasing the shear stress conditions inside the reactor or increasing the effluent recycling ratio affect the biogas profile inside granules when they lead to a reduction of the granule size [39] or to a better mixture in the reactor, by provoking the decrease of the substrate concentration in the bulk liquid. However, in some cases,

these strategies are useful to prevent the sludge bed from rising in the reactor but do not avoid the granules floatation caused by inner gas retention [39,40].

From previous experiences and the results from this analysis the best identified strategy to prevent floatation seems to stepwise increase the substrate loading rate applied to the system in order to maintain low substrate concentrations in the bulk liquid. The reduction of granule size seems to be a suitable strategy once floatation appears but such a procedure may disturb the microbial eco-system inside granules [9].

In order to define the operational zones where floatation is expected, the substrates concentrations which cause that N_2 or CH_4 concentrations inside the granules exceed their solubility value were defined for different granules sizes (Fig. 2). Values of granules size and substrate concentration reported in the literature to cause floatation events fit quite well inside the defined risk zones. In the case of denitrifying and methanogenic granular biomass systems fed with VFAs, scarce information is available about the substrate concentration and radius values to be used in the present work.

According to these calculations minimum values for the granule radius to prevent floatation can be estimated. In the case of the anammox process, granules with radius values lower than 0.038 cm should not float. This value is similar to that observed by Chen et al. [9] when characterized those granules able to settle during floatation episodes in an anammox reactor. For denitrification, granules with radius lower than 0.035 cm are not expected to float.

The previous calculations are applicable to granules where the single studied processes take place. However, in many cases granules comprise bacteria carrying out more than one biological process. As an example the previously performed calculations could be extrapolated to granules containing both ammonia-oxidizing and anammox bacteria to carry out autotrophic nitrogen removal. Since they generally operate at dissolved oxygen concentrations between 0.5–3.0 $mg\ O_2/L$, according to the ammonia oxidation stoichiometry, the increase of nitrite concentration in the outermost zone where anammox bacteria are present inside the granule is at most 0.1–0.9 $mg\ N-NO_2^-/L$. Therefore, the limit of substrate concentration to avoid granules floatation for this process can be considered almost the same as that calculated for Anammox process [41]. Nevertheless, each kind of granules performing more than one process must be studied separately to be sure that the specific issues are taken into account in the kinetic study.

3.2. Effect of temperature on granules floatation

With respect to the effect of the temperature its decrease causes the increase of the substrate concentration in the bulk liquid due to the decrease of the overall biomass specific activity (Fig. 3).

This effect is more pronounced the lower the granule size. For granules of 0.2 cm the overall biomass specific activity ($\eta \cdot r_{max}$) of anammox and denitrification processes is only slightly affected by temperature changes because the variations of the efficiency factor, mainly due to the variations of the substrate concentration, and r_{max} are similar in magnitude but with opposite tendencies. However, in the case of methanogenesis, there is clear effect of temperature on the overall biomass specific activity ($\eta \cdot r_{max}/K_S \cdot S$) since temperature has a higher effect on the kinetic parameters (r_{max}/K_S) than on the efficiency factor and substrate concentration.

Low temperatures promote the increase of substrate concentration in the bulk liquid but also a slight increase of products solubility (N_2 solubility: 18 mg/L at 20 °C and 15 mg/L at 35 °C; CH_4 solubility: 21 mg/L at 20 °C and 16 mg/L at 35 °C). In the case of granules with a diameter of 0.2 cm, the maximum theoretical N_2 concentration achieved inside the anammox and denitrifying

granules exceed its solubility value when the temperature is decreased down to 25 or 20 °C (Fig. 4A and B). Nevertheless, if the temperature is increased up to 35 °C, N₂ concentration inside the granules would be lower than its solubility value which would avoid the granules floatation. For the smallest particles the theoretical maximum N₂ concentration obtained at 25 or 20 °C does not exceed its solubility value. This fact can be attributed to the strong influence of temperature on the overall biomass specific activity for small granules (Fig. 3A1, B1 and C1). For this granules size, an increase in the temperature also favors the presence of low N₂ concentrations. Since anammox and denitrifying granules with diameters lower than 0.04 cm are not expected to float at the tested conditions (30 °C and 16 mg N-NO₂⁻/L and 25 mg N-NO₃⁻/L), then an increase in the operating temperature always leads to operational conditions that prevent the floatation of the granules. However, the decrease in the temperature could have a positive or negative effect depending on the granule size.

For anaerobic biomass, obtained results show that the decrease in temperature would also promote the floatation of granules with a diameter of 0.2 cm (Fig. 4C). However there is not a monotonous increase of CH₄ concentration inside granules with the temperature decrease since maximum CH₄ concentration achieved at 20 °C is lower than that achieved at 25 °C. For the smallest granules size, similar to what occurs in the case of anammox and denitrification processes, CH₄ concentration at 20 and 25 °C does not exceed its solubility value. Then, the decrease in the temperature is an advisable strategy to avoid the floatation phenomena only for the granules with the smallest size tested. Regarding the effect of a temperature increase, this action would reduce the risk of floatation for granules with diameters of 0.1 and 0.2 cm but its effect would be negative in the case of the smallest granules tested. Therefore, there is not a clear strategy to avoid granules floatation during the anaerobic process by changing temperature since its effect will depend on the granules size.

4. Conclusions

- Substrates concentration and granules size are the main parameters to be controlled during the operation of anammox, denitrifying and anaerobic systems in order to avoid floatation of biomass and its concomitant washout.
- To prevent floatation episodes, the loading rate applied to granular systems should not exceed their removal capacity in order to maintain the substrates concentrations lower than 16 mg NO₂⁻-N/L, 25 mg NO₃⁻-N/L and 130 mg COD/L (as acetate) for anammox, denitrifying and anaerobic systems operated at 30 °C, respectively.
- Once floatation occurs, to break-up granules is a feasible strategy to stop it since this action decreases the overall substrate consumption inside each granule, reducing the fraction of granule exposed to biogas desorption.
- Data obtained from the literature reporting floatation events fit into the identified zones with risk of floatation defined in the present study.
- The decrease of the operating temperature could avoid or promote floatation episodes depending on the granules size.

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