MODELLING SALINITY EFFECTS ON AEROBIC GRANULAR SLUDGE TREATING FISH-CANNING WASTEWATER

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KEYWORDS

Aerobic granular sludge; industrial wastewater; salinity; biokinetic model

ABSTRACT

The effect of salinity on aerobic granular sludge treating fish-canning wastewater was evaluated through a one-dimensional biofilm model. Salt inhibition of heterotrophic and nitrifying bacteria was described by a non-competitive inhibition term, for which the value of the inhibition coefficient was estimated based on data from literature. The model was calibrated and validated with experimental lab-scale data regarding COD and nitrogen removal from

industrial wastewater. Two dynamic operating periods with salinities of 13 and 5 g NaCl/L were used for calibration and validation, respectively. The prevailing feast-famine regime necessitated simultaneous growth and storage processes to accurately describe COD removal. The presence of salt caused nitrite accumulation, as well as unusually low estimated maximum growth rates of nitrifying bacteria. The addition of a salinity inhibition term to the model could accurately describe the COD and nitrogen species experimentally measured along the cycles with different salinities.

1. INTRODUCTION

The Aerobic Granular Sludge (AGS) technology is nowadays regarded as an established way to treat both municipal and industrial wastewater, resulting in significant space and energy savings compared with traditional activated sludge processes [1]. In order to profit from these benefits, there has been an increasing interest to also treat saline wastewater with AGS. This includes Wastewater Treatment Plants (WWTP) from urban areas located in coastal areas, which have infiltrations of saline groundwater [2,3], as well as from different industrial sectors, such as the petrochemical industry [4] or seafood processing [5,6].

High salt concentrations affect the reactor performance through their effect on both the physical properties of the granules and biological activity. Overall, saline wastewater, with moderate salt concentrations (5 – 15 g NaCl/L), can be beneficial for the formation of dense and smooth aerobic aggregates with good settling properties [7]. The presence of salt provokes an increase of the bulk liquid density and leads to the washout of light and poor settling aggregates [8]. As a consequence, there is a reduction of the biomass concentration in the system, where only the dense aggregates with good settleability are present. In addition, the growth of granules with a regular and smooth surface is promoted and the production of extracellular polymeric substances is increased [9,10].

However, high salinity has been reported to have a detrimental effect on biological activity. Different inhibition thresholds have been observed depending on (1) the tolerance of each type of bacteria and (2) the adaptation of the biomass to high salt concentrations. The inhibitory effect of salinity has been studied mostly on nitrifying bacteria, whereas less research works paid attention to salt inhibition of heterotrophic bacteria. In general, salt concentrations lower than 10 g NaCl/L do not significantly affect the biological activity. However, when the salt content is above 10 g NaCl/L, the biological activity is usually reduced [11]. For example, batch tests performed by Bassin et al. [8] showed a complete inhibition of the ammonium uptake rate at a salt concentration of 20 g NaCl/L without previous acclimatization of AGS to salinity. Li et al. [10] observed an almost complete inhibition of NOB with salinities higher than 6 g NaCl/L. Moreover, Wang et al. [11] reported an important AOB activity reduction (20% of the initial value) with salt concentrations above 15 g NaCl/L. If the biomass is adapted to salinity, both AOB and NOB are usually able to withstand higher salt concentrations. For example, Bassin et al. [8] and Pronk et al. [12] reported complete inhibition of salinity-adapted NOB with a salt concentration of 33 g NaCl/L and 20 g Cl^-/L, respectively. Most of the studies agree that NOB are more sensitive than AOB to high salt concentrations [8,12,13]. However, a few have reported the opposite behaviour [11,14].

Modelling is a useful tool to gain understanding in and to optimize the biological processes involved in wastewater treatment. The Activated Sludge Models (ASM) have been used to simulate the bioconversion processes that occur in AGS reactors. Depending on the specific reactor configuration, different biological processes take place, and thus, different ASM models are used [15]. Aerobic granular sludge reactors removing organic carbon, nitrogen and phosphorus are best described with ASM2-ASM2d models, which consider Phosphate Accumulating Organisms (PAO) activity [16]. On the other hand, in AGS reactors with a short feeding phase, only organic carbon and nitrogen conversion processes take place. In this case,

ASM1 and ASM3 [16] are sufficient to model the reactor performance. Although ASM models have been successfully applied to simulate many case studies [15], none of them has addressed the treatment of saline wastewater and explicitly considered the salinity effect on the biological activity of AGS.

In this study, a model was set up to describe the operation of an AGS treating industrial saline wastewater. Several research objectives were defined for this purpose. First, the best approach to describe the biological conversions was evaluated by comparing alternative models against each other (ASM1 versus ASM3). Second, the need to consider an inhibition term to include the effect of salinity was investigated. Third, the value for the inhibition constant was estimated based on literature data. Finally, the model was calibrated and validated under dynamic (cyclic) reactor operation, treating influent with different salt concentrations.

2. MATERIALS AND METHODS

2.1. Experimental data

The operating conditions of an AGS reactor fed with saline wastewater from a fish-canning industry, described by Carrera et al. [7], were taken as a reference scenario. The reactor had a working volume of 1.7 L and a volume exchange ratio of 50%. The feeding strategy consisted of pulse feeding followed by aeration (no anaerobic feeding). The length of the operational cycles was 4 h, distributed as follows: 5 min of feeding, 227 min of aeration, 1 min of settling and 7 min of effluent withdrawal. During the aeration phase, the dissolved oxygen concentration reached saturation values of 8.6 mg O_2/L. The reactor operation was divided into different phases, corresponding to different batches of wastewater (Figure 1). The changes in wastewater composition (salt, COD and nitrogen concentration) were associated to the seasonal variations of the processed fish products in the factory - no adjustments were made by the authors.

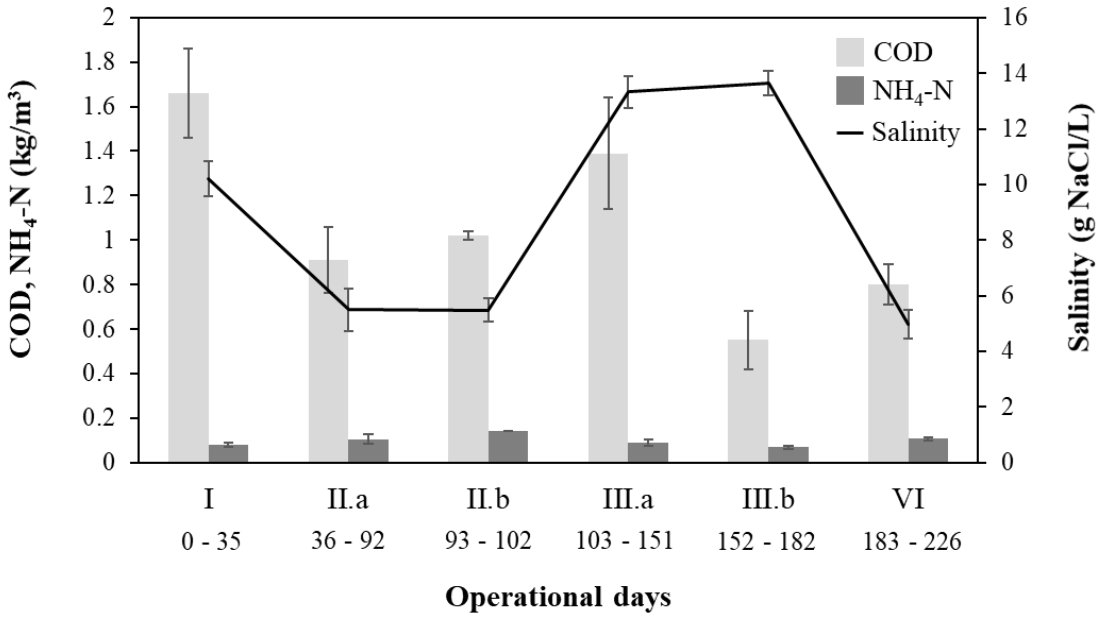


Figure 1: Influent characterization of the AGS reactor in terms of soluble COD, ammonia and salt concentration. Provided data are average values plus standard deviation corresponding to the analytical measurements performed in each phase.

The experimental data selected for model calibration and validation corresponded to two operational phases where the reactor was operating in steady-state conditions (fully-granular system, stable nitrifying and heterotrophic activity). In particular, the dataset corresponding to phase III.b was selected for calibration, since the reactor was operated with the highest salt concentration (13 g NaCl/L). Relatively constant biomass concentration and effluent composition were observed during the entire stage. Thus, it was considered to be at steady-state conditions [7]. For validation, the experimental data from phase IV was used, with an influent salt concentration of 5 g NaCl/L. In this stage, biomass washout occurred due to the decrease of salinity, but the effluent concentrations were relatively constant. Detailed information related to the operational conditions of the reactor used as input of the model is presented in Table S.1. More detailed information about the reactor operation can be found in Carrera et al. [7].

3. AEROBIC GRANULAR SLUDGE REACTOR MODEL

3.1. Bioconversion models

Both ASM1 and ASM3 [17] were applied to describe the organic carbon and nitrogen conversions in the AGS reactor. Both models include the growth and decay processes of heterotrophs and autotrophs, and the hydrolysis of organic matter and nitrogen compounds. However, ASM1 considers direct biomass growth on substrate, whereas ASM3 assumes that substrates are first stored and biomass growth subsequently takes place on storage polymers.

Two modifications were made for an accurate description of the biological processes in the AGS reactor under study. The first modification of the model was the description of nitrification as a two-step process, involving the oxidation of ammonia to nitrite by AOB, and the subsequent oxidation of nitrite to nitrate by NOB, as done in previous studies [18,19]. Nitrite was also included as an intermediate compound in the denitrification processes. This modification is especially important when treating saline wastewater, since in many cases NOB can be inhibited due to the presence of salt. ASM3 with the abovementioned modifications has been successfully applied in a few studies modelling AGS reactors [20–24]. This modification was applied to both ASM1 and ASM3.

The second modification was the introduction of simultaneous growth and storage by heterotrophic bacteria, as proposed by Krishna and Van Loosdrecht [25]. Indeed, in systems with pulse feeding followed by aeration (aerobic feast-famine), both storage and growth take place at the same time under feast conditions. So, in order to provide an accurate description of the process, direct biomass growth on soluble COD was included in the model, in addition to growth based on stored polymers. This modification was applied only to ASM3, since ASM1 does not include storage processes.

The developed model included 7 soluble and 5 particulate compounds (Table S.2). The soluble compounds were dissolved oxygen (S_O), soluble easily biodegradable COD (S_S), soluble inert COD (S_I), organic nitrogen (S_{ND}), ammonium (S_{NH}), nitrite (S_{NO2}) and nitrate (S_{NO3}). The particulate compounds comprised: particulate slowly biodegradable COD (X_S), particulate inert COD (X_I), heterotrophic bacteria (HB, X_H), storage compounds (X_{STO}), AOB (X_A), and NOB (X_N). Alkalinity was not included in the model because it was not a limiting process parameter. In addition, pH and temperature were considered constant, due to the low fluctuations during each operational phase of the SBR operation (7.2 – 7.5 and 22.6 – 23.8ºC for pH and temperature, respectively). The soluble COD concentration (S_{COD}), calculated as the sum of soluble inert COD (S_I) and soluble easily biodegradable COD (S_S), was included in the model as an additional output variable, for comparison with the experimentally-measured soluble COD concentration. The stoichiometric matrix of soluble and particulate compounds is presented in Table S.3. All bioconversion reactions included in the model are listed in Table S.4.

3.2. SBR operation

To describe the discontinuous operation of the SBR in Aquasim, the total reactor volume was divided into a biofilm reactor compartment and a mixed reactor compartment. They were coupled with a diffusive link (exchange coefficient 1000 m^3/h) to let the liquid phase behave as one perfectly mixed water volume and ensure the same bulk liquid concentration in both compartments [26]. The volume of the biofilm compartment was fixed at 0.25 L. It was defined as a confined reactor type with rigid biofilm matrix. The completely mixed reactor contained the remaining liquid, and had a variable volume. The maximum value was 1.7 L during the aeration phase.

158 The transport processes inside the granules, which are influenced by diffusion coefficients,
159 density and porosity were described through Aquasim. Mass transfer resistance from the bulk
160 liquid to the granule surface was neglected. To model the intragranular transport, a compound-
161 specific estimation of the effective diffusion coefficient inside a biofilm matrix was used [27].
162 The granule depth was divided into 20 grid points.

163 **3.3. Description of salinity effects**

164 The effect of salinity on biological activity was considered as a non-competitive inhibition.
165 Different terms have been proposed in literature to describe this type of inhibition in bacteria
166 (Table S.6). In this study, the inhibition term was added to the growth processes of all the types
167 of bacteria (AOB, NOB and heterotrophs).

$$\frac{K_{Inh,50}}{K_{Inh,50} + S_{Inh}} \quad \text{(Eq.1)}$$

168 The value of the half-saturation constants was estimated based on activity tests of different
169 research works, that report a reduction of the biological activity with the increase of salinity.
170 Different parameter values were obtained for biomass non-adapted or adapted to salinity (Table
171 1). A detailed description of the estimation of this parameter is provided in the results and
172 discussion section.

173 Table 1: Estimated values for the saturation constant ($K_{Inh,50}$) of the inhibition term ($\frac{K_{Inh,50}}{K_{Inh,50}+S_{Inh}}$).

Bacteria	**$K_{Inh,50}$ (g/L)**	**Salinity adaptation**
AOB	10.7 ± 3.2	No
	24.8 ± 9.5	Yes
NOB	13.5 ± 0.0	No
	21.7 ± 5.5	Yes
HB	23.0 ± 0.0	No
	44.3 ± 10.4	Yes

174

3.4. Simulation set-up

Simulations were done for constant influent composition, corresponding to the measured average values of each operational phase (Table S.1). The simulations were run with the reference operational conditions (Table S.1) for 200 days to ensure a steady-state operation and microbial population distribution. The operation was considered at stationary state conditions when the effluent concentrations profiles of all compounds were the same (within less than 5% difference with respect to the final value) in at least two consecutive cycles and also the spatial profiles of all biomass types inside the granule remained unchanged. The model was implemented in the Aquasim simulation software [28].

To calibrate the model, the simulation was first run with default values of the kinetic parameters, provided by ASM1 and ASM3 [16]. The COD, NH_4-N, NO_2-N and NO_3-N profiles obtained with the model were compared with experimental data from a representative cycle per operating phase, corresponding to the conditions used as input of the model. Model calibration was performed sequentially, firstly focused on COD removal, and afterwards nitrogen removal, as suggested by Rittman et al. [29]. To do that, the half-saturation coefficients were maintained, whereas the maximum growth rates were modified.

4. RESULTS AND DISCUSSION

4.1. Evaluation of ASM1

The SBR operation was first simulated based on ASM1, to evaluate what could be achieved with a relatively simple model (compared to ASM3). To fit the experimental data (from phase III.b) in terms of COD conversion rate, the maximum growth rate of heterotrophic bacteria was estimated at the maximum of the tested range, namely $\mu_{max,H} = 20\ d^{-1}$ (Figure S.2). This value seemed unreasonably high compared to ASM1 and ASM3 models describing the operation of

AGS reactors, which were in a range of 2 [16] – 13 d^{-1} [30]. Therefore, ASM1, in which COD removal is only associated to biomass growth, did not provide an accurate description of the biological processes occurring inside the reactor for organics removal. Since it was not possible to reproduce the same COD conversion rates of the experimental data, ASM1 was discarded and subsequent ASM3 implementation was tested.

4.2. Calibration of ASM3

Using ASM3, it was possible to reproduce the COD and N conversion rates observed experimentally. This indicates the need to consider the storage processes, in addition to biomass growth, to explain the COD conversion rates. For this reason, ASM3 (and not ASM1) is the most suitable model to simulate the performance of an AGS reactor with short feeding phase. At this point, salinity inhibition was not yet explicitly considered in the model, in order to describe the observed reactor performance with the simplest model possible. More specifically, it was evaluated whether the estimated kinetic parameters were in a realistic range.

The results of the ASM3 calibration showed the consumption of all biodegradable COD in the first minutes of the cycle, during the feast phase (Figure 2.a). The COD present at the end of the cycle was attributed to the inert fraction (fractionation tests showed a S_I concentration of 170 g COD·m^{-3}, see Table S.1). Ammonia was completely oxidized to nitrite, whereas further oxidation to nitrate did not take place (nitratation activity not detected) (Figure 2.b). This agreed with the experimental findings, where the presence of nitrate and NOB were measured, but not detected due to salt inhibition (more details in Carrera et al. [7]). In addition, during the first minutes of the cycle, denitritation of nitrite from the previous cycle took place.

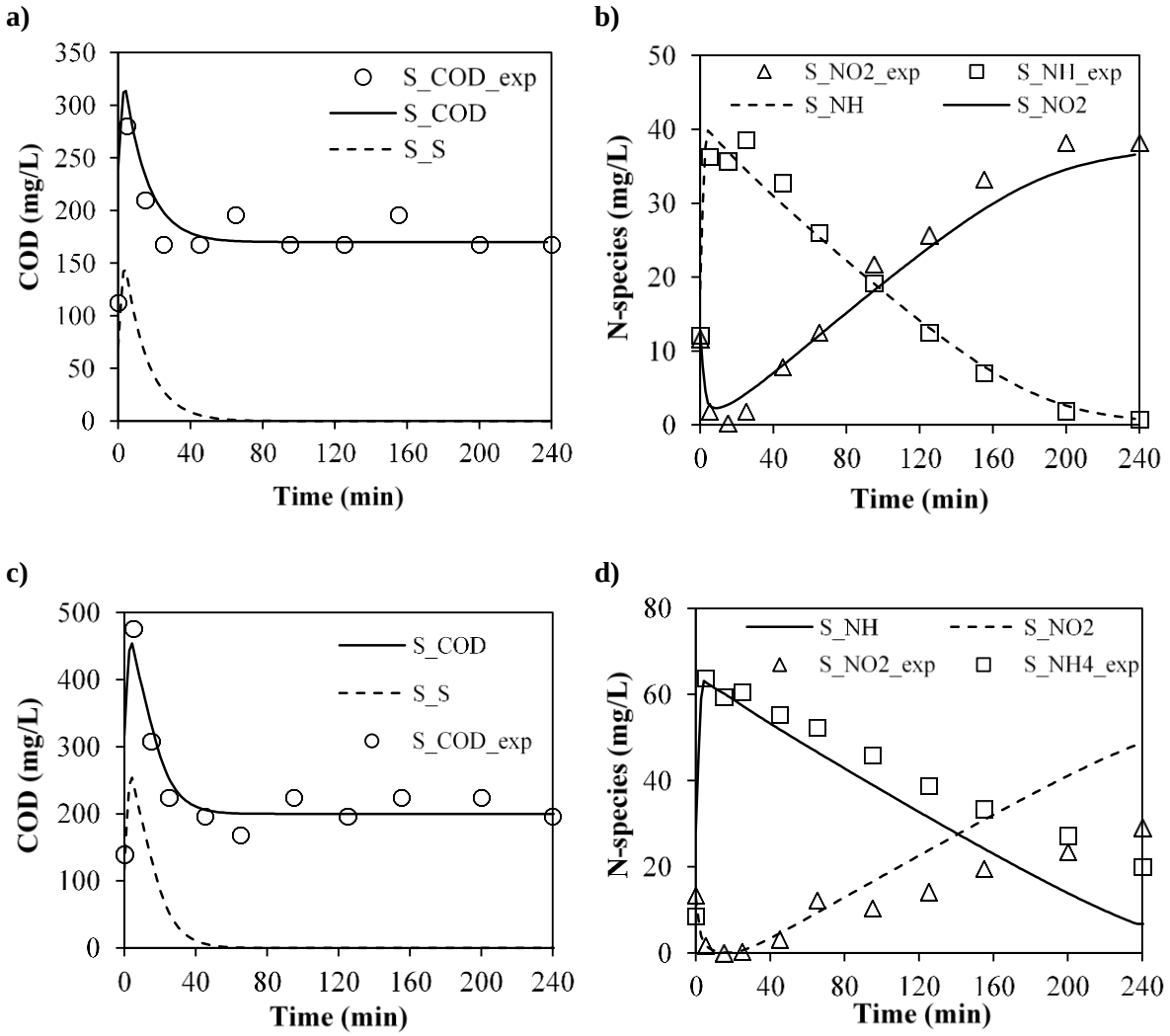
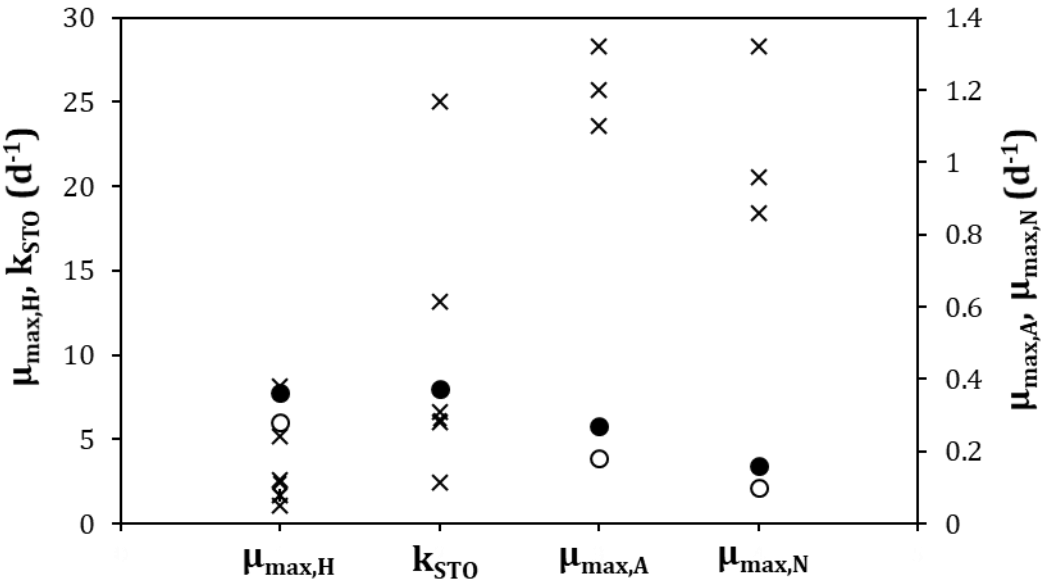


Figure 2: Results of the ASM3 model calibration (a,b) and validation (c,d). Cyclic concentration profiles in the AGS for (a,c) S_{COD} and S_S and for (b,d) S_{NH} and S_{NO2}. Markers represent experimental data; full lines denote simulation results.

Heterotrophic bacteria (X_H) were present in the external layers of the granule, whereas autotrophs (X_A) were located in inner layers of the granule, due to the lower growth rates compared to heterotrophs (Figure S.3). There was a progressive increase of the inert material (X_I) in direction to the centre of the granule, which indicates an important fraction of the granule which is not active.

The calibrated heterotrophic bacteria kinetic parameters ($\mu_{max,H}$ of 6 d^{-1} and k_{STO} of 8 d^{-1}) were similar to those from previous research works modelling AGS with ASM3 (Figure 3).



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232 Figure 3: Comparison of the kinetic parameters of this model (with (●) and without (○) adding the salt
233 inhibition term) with the values obtained in other studies (x) modelling AGS with ASM3 [16,20–24].

234 However, the calibrated values of nitrifying bacteria ($\mu_{max,A}$ of 0.18 and $\mu_{max,N}$ of 0.10 d^{-1})
235 were lower than the reported ranges of 1.1 – 1.3 and 0.9 – 1.3 d^{-1} for $\mu_{max,A}$ and $\mu_{max,N}$,
236 respectively [20–24].

237 These low values could be attributed to a number of reasons. First, one of the possible causes
238 for low values of $\mu_{max,A}$ and $\mu_{max,N}$ may be a high biomass retention inside the reactor (in this
239 study the Sludge Retention Time (SRT) was 10 – 15 d). Chiellini et al [31] estimated the value
240 of $\mu_{max,A}$ in a Conventional Activated Sludge (CAS) reactor and a Membrane bioreactor
241 (MBR). They observed a lower value of 0.46 d^{-1} in MBR, with a SRT of 20 days, whereas in
242 CAS it was of 0.96 d^{-1} (SRT below 10 d). Munz et al. [32] also established a comparison
243 between CAS and MBR and observed the same results, a $\mu_{max,A}$ of 0.35 d^{-1} in an MBR and of
244 0.72 d^{-1} in CAS. They pointed out that the cause could be the difference between the SRT of
245 both systems (20 d in the MBR, 8 d in the CAS). In systems with biofilms the higher biomass
246 retention and SRT promote the selection of slow-growing nitrifiers, which present low values
247 of μ_{max}. The SRT values of the AGS reactor in this study are more comparable to MBR systems,

for this reason the development of slow-growing nitrifiers (low values of μ_{max}) is favoured [31, 32].

Another factor that could reduce the value of $\mu_{max,A}$ is the salt concentration inside the reactor (in this study the salt concentration fluctuated between 5 – 13 g NaCl/L). Cui et al. [33] observed that the increase of the salt concentration (30 – 85 g NaCl/L) stimulates the growth of halophilic nitrifiers, characterised by a high affinity for ammonium and low growth rates (low half-saturation constant and low μ_{max}). They determined a μ_{max} of 0.26 d^{-1} with a salt concentration of 30 g NaCl/L and half-saturation coefficient values of 1 – 1.7 mg NH_4^+-N/L. The kinetic parameters showed a decreasing trend with the increase of salinity. They suggested the possibility of a shift in the community composition due to a long-term salinity selection. Gonzalez-Silva et al. [34] also observed a change with time of the microbial community composition as a response to the variations on salt concentration and its adaptation to saline conditions. Although the salt concentration tested in the current study (5 – 13 g NaCl/L) was lower than in the abovementioned previous works (30 – 85 g NaCl/L), the inoculum of the reactor came from a biological reactor of a fish cannery, adapted to even higher salinity, so the nitrifying bacteria might be different populations compared to an inoculum non-adapted to salinity.

Additionally, a few studies have also reported values of $\mu_{max,A}$ lower than those in ASM3. Gao et al. [35] obtained a value of 0.46 d^{-1}. They explained that it was due to the shortcut nitrification process, with only nitrite production. The biomass production, in this case, was less than in the conventional nitrification process considered in ASM3 (AOB+NOB activity in a single process). Consequently, the value of μ_{max} was lower than ASM3.

In this study, the calibrated values ($\mu_{max,A}$ of 0.18 and $\mu_{max,N}$ of 0.10 d^{-1}) obtained in this study are still somewhat lower than those from literature (0.46 [31,35], 0.35 [32], 0.26 [33] d^{-1}). This

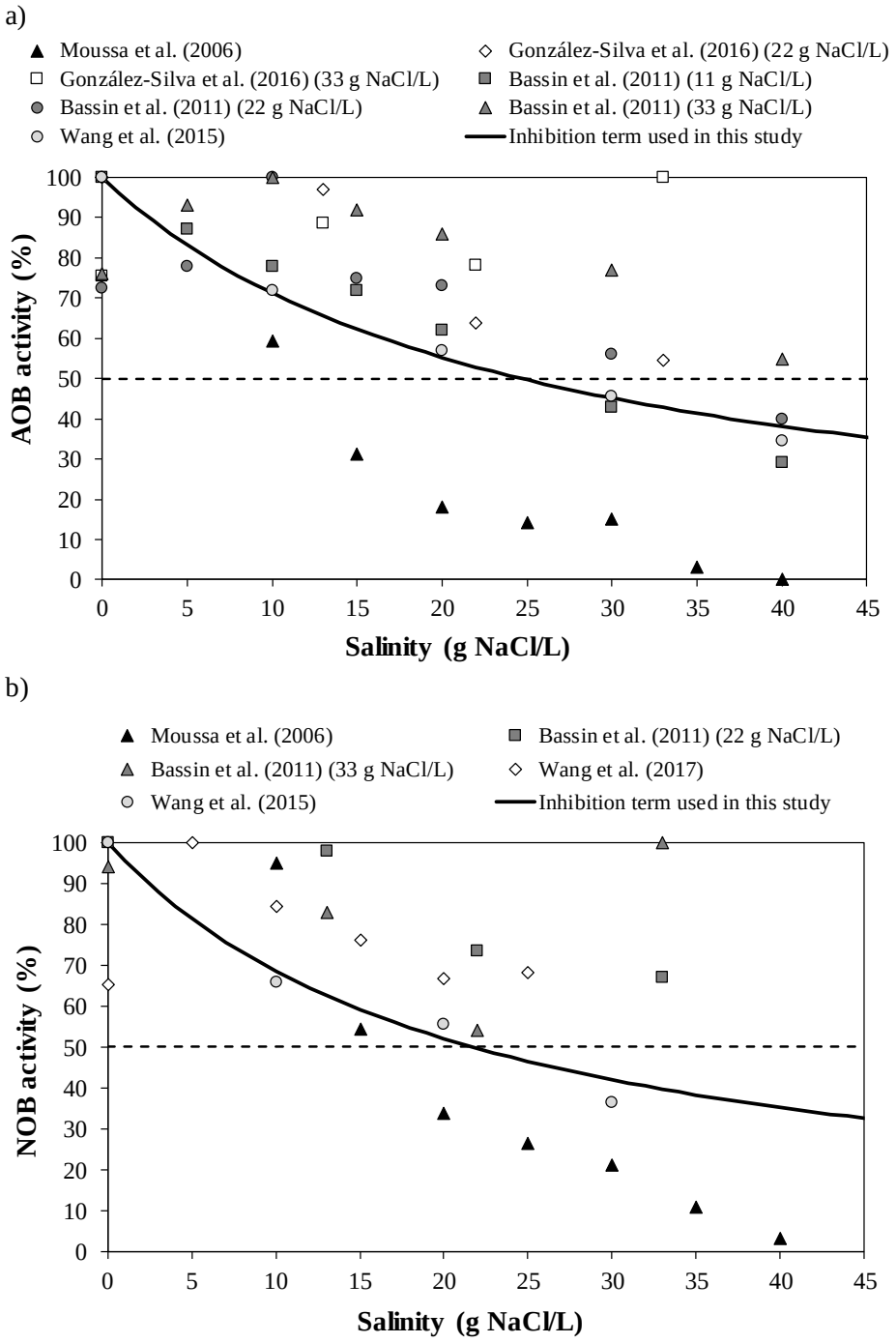
could be due to the combination of the three causes mentioned before (SRT of 15 days, salinity of 13 g/L and only nitritation).

4.3. Addition of salinity effects

Since salinity was likely one of the causes for the low values of the maximum growth rates, the salinity inhibition term was added to the model to explicitly consider its effects on the different bacterial populations. This allowed the comparison between the maximum growth rates of this study with other research works regardless of the salt concentration (Figure 3).

To estimate the salinity inhibition constant of each bacterial population, firstly a literature review was made about the reported decrease of the biological activity of biofilms (mainly AGS) with the increase of salinity. The gathered data was divided into two groups: biological activity reduction of (1) biomass adapted and (2) non-adapted to salinity. In this study, data from group (1) (Figure 4) was used to estimate the inhibition constant, since the cultivated AGS was adapted to the presence of salt. Additionally, data corresponding to non-adapted biomass (not used in this study) can be found in supplementary materials (Figure S.1). The activity reduction of the different types of bacteria from different studies was plotted. Then, the half-saturation constant was estimated by reading in the figures the salt concentration corresponding to the 50 % decrease of the maximum activity (dashed line).

The inhibition constant of heterotrophic bacteria was determined based on values reported in literature [36] due to the lack of specific activity assays at different salt concentrations. This study reports the reduction of the biological activity of AGS with the gradual increase of salinity in a sequencing batch reactor.



293

294 Figure 4: (a) AOB activity and (b) NOB activity with different salt concentrations of biomass adapted
295 to salinity.

296 With the addition of the salinity term to the model, the maximum growth rates were re-
297 calculated to provide the same results as Figure 2 (a,b). The calibrated values were higher than
298 in the previous section: 0.27, 0.16 and 7.76 d^{-1} for AOB, NOB and heterotrophs, respectively
299 (Figure 3), while the conversion rates were the same. This indicates that the addition of the

inhibition term could reproduce the experimental findings. Regarding the biological activity inhibition, heterotrophic bacteria were the least affected by the presence of salt. The COD conversion rate was reduced 23 and 10 % under salinity conditions corresponding to calibration and validation, respectively. AOB and NOB activities suffered higher reductions, especially with the salt concentration of 13 g NaCl/L (calibration, 34 and 37% activity reduction for AOB and NOB, respectively), showing a higher sensitivity of nitrifying bacteria to the increase of salinity.

4.4. Model validation

In the validation step, the results of the model (the salinity inhibition term was included) were compared with an operational cycle at a salinity of 5 g NaCl/L. The COD consumption was accurately reproduced (Figure 2,c). However, the predicted ammonia oxidation was faster than the experimental data (Figure 2,d). The ammonia and nitrite concentration of the effluent were underestimated and overestimated respectively, both in a 65 % (calculated as the difference between the experimental and the model value, divided by the experimental value). This could be due to the fact that the experimental cycle measurement was done in a moment when the reactor was not working in steady-state conditions. A few days before the cycle measurement (days 196 – 209), the washout of part of the biomass took place, associated to the change of salinity [7]. Although a stable effluent quality was achieved in these days, the biomass might have been still adapting to the new conditions of the reactor.

In order to validate the model with experimental data from steady-state conditions, the effluent quality predicted by the model was also compared with the effluent quality measured experimentally during phase IV before the washout of the biomass (Figure 5). The results indicate that, despite the different conversion rates of AOB, the model was able to predict the effluent concentrations of both organic matter and nitrogen compounds.

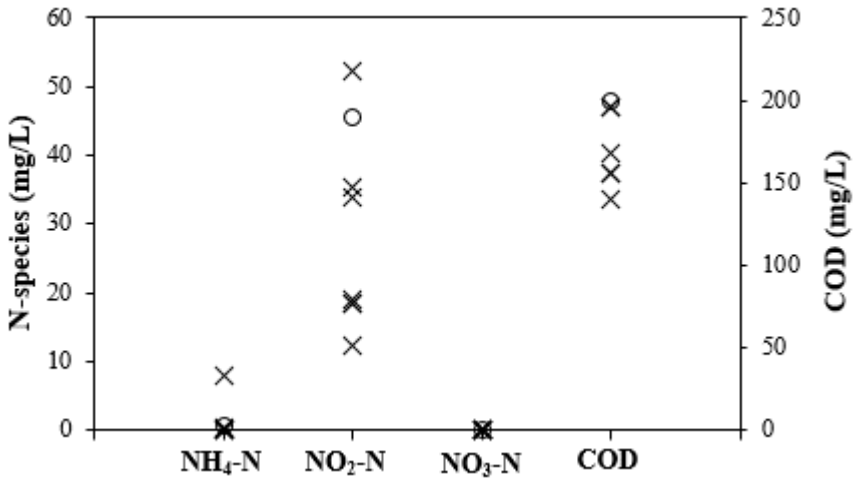


Figure 5: Comparison of the effluent quality from the experimental data of phase IV (x) and predicted by the model (○).

The biokinetic model considering the inhibition of salinity on the biological activity was calibrated with a representative cycle measurement from an operational phase of an AGS reactor with 13 g NaCl/L. In addition, it was validated with the effluent measurements of a different phase, with 5 g NaCl/L. However, the model has some shortcomings. It was observed experimentally that salt fluctuations not only affected the biological activity, but also the physical properties of the biomass [7]. In this model, only the effect of salinity on the bioconversion processes was considered. In addition, the salt concentrations used as input of the model were low-moderate, and the model should be tested with higher concentrations (20 – 40 g NaCl/L). Further research is needed to (1) test the biological model with a wider range of salt concentrations to assess the impact of salinity on the biological activity and (2) study the feasibility of incorporating also the effect of salinity on the physical properties of the biomass. Additionally, further research is needed to clarify what is exactly the effect of salinity on the bacterial populations: inhibition, shift of bacterial populations or a combination of both. This will further help to determine if an inhibition term is sufficient for a proper description of reactor performance or if it is more important to re-calibrate the growth rates according to the specific bacterial communities present in the reactor. This model could be further used as a

343 starting point for future research. For instance, the model could be applied in the design stage
344 of future AGS reactors or for system optimization and scenario analysis of existing reactors
345 treating saline wastewater.

346 **5. CONCLUSIONS**

347 In this study, an ASM3-based biofilm model was demonstrated suitable to describe the
348 performance (COD and N conversions) of an AGS reactor with a short feeding phase treating
349 industrial saline wastewater. ASM1 could not predict the COD conversion rates observed
350 experimentally, which underlined the importance of explicitly describing substrate storage.

351 Model calibration revealed unusually low growth rates for nitrifying bacteria, which was
352 mainly attributed to the effect of salinity, combined with other factors such as a high SRT. The
353 presence of salt also provoked nitrite accumulation. The addition of a non-competitive
354 inhibitory term to the model could accurately predict the COD and nitrogen conversions in the
355 AGS reactor under different salinities.

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