Decay processes of AOB in ASM including protozoa grazing

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Abstract. Protozoa grazing of nitrifiers is important process in activated sludge process that leads to decrease of mass of active bacteria. In classical models this process is included in decay processes. Such approach makes modelling of influence of protozoa grazing on nitrifiers impossible. In cases when maximization of nitrifiers growth is important (such as nitrifiers cultivation for bioaugmentation) this simplification becomes major drawback. This paper presents extended version of ASM3 model that includes protozoa grazing as well as results of calibration of protozoa grazing process on experimental data. Additionally new approach for analysis of decay rate is proposed. This approach includes calculation of new parameters: substitute decay rate which is sum of ASM3 base decay rate and excluded predation process.

1 Introduction

Protozoa are common component of activated sludge with numbers in order of 3-20x10⁶ cells·l⁻¹ [1] and dry weight of even 250 mg·l⁻¹ [2]. Protozoa grazing is well known phenomenon that has influence on bacteria community in activated sludge [1]. Presence of protozoa triggers floc formation as bacteria in flocks are less prone to consumption. Protozoa consumes suspended bacteria and therefore leads to better effluent quality [1]. In extreme situation protozoa may however cause process collapse [3]. It is assumed that protozoa are responsible for higher decay rate in aerobic conditions in comparison to anoxic or anaerobic ones as protozoa are strict aerobes and graze only in presence of oxygen [4].

In classical models, protozoa grazing is included in decay processes [5] which makes separate analysis of protozoa influence impossible. However separate modelling of protozoa grazing is in early development with few research articles available [6, 7, 8]. Distinct feature of these early approaches to separate modelling is that protozoa grazing is added to classical models but rate of decay processes is not decreased in parallel. Therefore protozoa grazing is included doubly: firstly in decay rate and secondly in separate process.

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1.1 The problem and its importance

As protozoa grazing has identical result as other decay processes i.e. loss of bacterial mass, this approach leads to underestimation of influence of protozoa as model can be calibrated only when protozoa are small in number and consume low amount of bacteria. Additionally, it is difficult to compare models including separate protozoa grazing with classical ones and check whether new approach leads to similar mass loss of bacteria as classical ones. These problems may cause wrong calibration of models and false results.

Proper modelling of grazing is important especially in case of ammonia oxidizing bacteria (AOB) as that group is low in mass and modelling of their population is important in many technologies such as bioaugmentation, partial nitrification, deammonification where maximization of their mass is of major importance.

1.2 Solution to problem

Proposed solution for decay rate problem mentioned above is (fig 1.):

- aerobic decay rate of AOB is lowered to value in accordance with typical anoxic decay rates.
- higher decay rate in aerobic conditions is due to activation of protozoa.
- substitute decay rate which is sum of classical decay rate and mass load due to grazing is proposed.

Substitute decay rate is a tool to compare calibration results with values of classical decay rates from literature to check whether compliance is achieved. As protozoa grazing is normally included in decay rate, compliance between classical values of decay rates and sum of new decay rate and influence of protozoa must be achieved.

Fig. 1. Solution for decay rate problem.

1.3 Aim and scope of paper

Aim of paper is to propose new approach to decay process of AOB that includes lower decay rate and new parameter (substitute decay rate) and to show that proposed solution enables proper calibration of model and to show that obtained value of substitute decay rate is in accordance with classical values.
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1.2 Solution to problem

Proposed solution for decay rate problem mentioned above is (fig 1.):

- Aerobic decay rate of AOB is lowered to value in accordance with typical anoxic decay rates.
- Higher decay rate in aerobic conditions is due to activation of protozoa.
- Substitute decay rate which is sum of classical decay rate and mass load due to grazing is proposed. Substitute decay rate is a tool to compare calibration results with values of classical decay rates from literature to check whether compliance is achieved.

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Scope of studies designed for fulfilling aim of paper is presented on fig. 2. Three first stages are presented in materials and methods, while calibration of model is presented in results.

Fig. 2. Scope of studies.

2 Materials and methods

2.1 Model development (stage 1)

Computer simulations were made based on ASM3 model [5] extended with two step nitrification and denitrification processes [9] and protozoa predation [7].

Considering lack of data from literature concerning dynamics of protozoa predation on all components of activated sludge, authors implemented modifications to better represent these processes including all types of bacteria \(X_B = X_H + X_{AOB} + X_{NOB}\). All changes resulting from including protozoa \(X_{pred}\) in model equations have been listed below:

- Predation has been considered as a single process for overall concentration of biomass \(X_B\) in the reactor. Loss of each bacteria group \(X_H, X_{AOB}, X_{NOB}\) is based on a statistical model, meaning predation on particular bacteria group is proportional to its share in \(X_B\). (According to authors current knowledge such approach is the most reasonable from nutritional preference of protozoa).
- Anoxic decay of protozoa has been added.
- Stoichiometry of biomass loss resulting from protozoa predation has been modified with regard to ammonium release during these processes. Authors assumed it to be two step process – during predation and during protozoa decay.
- Reduction factor for anoxic endogenous respiration has been omitted for bacteria, due to assumption that decay in anoxic conditions is decreased because of lack of protozoa activity.

Implemented protozoa predation is similar to what Ni et al. [7] has proposed and depends on predation rate \(k_{pred}, 1/d\), dissolved oxygen concentration \(S_O, gO_2/m^3\), predators oxygen affinity concentration \(K_{O,pred}, gO_2/m^3\) as well as solid affinity constant for predators \(K_X, gCODx/m^3\) and overall concentration of biomass \(X_B, gCODx/m^3\).

Predators decay processes are analogical to autotrophic bacteria endogenous respiration proposed by Kaelin et al. [9]. Decay coefficient for predators \(b_{pred}, 1/d\) in anoxic conditions is additionally multiplied by reduction factor \(\eta_{pred}\) and dependent of nitrate concentration \(S_{NO_3}, gN/m^3\) and nitrate affinity constant for predators \(K_{NO_3,pred}, gN/m^3\).

Added processes are presented in table 1.
Table 1. Added kinetic expressions for predation process.

| Process                                      | Equation                                      |
|----------------------------------------------|------------------------------------------------|
| 1. Protozoa predation on X_B                 | \[ k_{pred} \frac{S_0}{K_{O,pred} + S_0} X_B \] |
| 2. Aerobic decay of X_pred                   | \[ b_{pred} \frac{S_0}{K_{O,pred} + S_0} \]   |
| 3. Anoxic decay of X_pred                    | \[ b_{pred} \eta_{pred, end} \frac{K_{O,pred}}{K_{O,pred} + S_0} X_{NO3} \] |

Description of symbols used in table 1: X_B - overall concentration of biomass; X_{pred} - concentration of protozoa; k_{pred} - predation rate; S_O - dissolved oxygen concentration; K_{O,pred} - oxygen affinity constant for protozoa; K_X - biomass affinity constant for protozoa; b_{pred} - decay coefficient for predators; \eta_{pred} - anoxic reduction factor for predators; S_{NO3} - nitrate concentration; K_{NO3,pred} - nitrate affinity constant for predators.

Growth of predators depends on yield coefficient for predators (Y_{pred}, gCOD_{pred}/gCOD_X) and fraction of produced inert COD generated during this process (f_i).

Mentioned above two step release of ammonium (S_{NH}, gN/m^3) is outcome of ammonium content in biomass (i_{NBM}) and in generated inert fraction (i_{NXi}). These relations have been presented in table 2.

Table 2. Stoichiometric matrix for modified model including predation on multiple bacteria fractions.

| Process | S_{NH} | X_H | X_AOB | X_NOB | X_pred | X_1 |
|---------|--------|-----|-------|-------|--------|-----|
| 1       | i_{NBM}(1 - Y_{pred}) - f_i i_{NXi} | - \frac{X_H}{X_B} | - \frac{X_{AOB}}{X_B} | - \frac{X_{NOB}}{X_B} | Y_{pred}(1 - f_i) | f_i |
| 2       | i_{NBM}Y_{pred} - f_i i_{NXi} | -1 | | | | f_i |
| 3       | i_{NBM}Y_{pred} - f_i i_{NXi} | -1 | | | | f_i |

Description of symbols used in table 2: S_{NH} - ammonium concentration; X_H - heterotrophs concentration; X_{AOB} - ammonia oxidizers concentration; X_{NOB} - nitrite oxidizers concentration; X_1 - inert particular organic material concentration; i_{NBM} - ammonium content in biomass; i_{NXi} - ammonium content in inert fraction; Y_{pred} - yield coefficient for predators; f_i - inert fraction generated during decay processes.

2.2 Substitute decay rate (stage 1)

Decay rate in ASM3 model consists of several components such as maintenance, endogenous respiration, lysis, motility, death as well as predation. With excluding the last one from this coefficient and presenting it as a separate function substitute decay rate (b_{asub}, 1/d) has been proposed (equation 1), which is a sum of base endogenous respiration rate of ASM3 model and predation process on corresponding bacteria biomass. Because of the lack of protozoa activity in anoxic conditions substitute decay rate has been presented for aerobic conditions alone. To prevent doubling the process of predation base endogenous decay rate has been decreased.
\[ b_{a,sub} = b_a \left( \frac{S_{O2}}{S_{O2} + K_{O2,a}} \right) + k_{pred} \left( \frac{S_{O2}}{S_{O2} + K_{O2,pred}} \right) \left( \frac{x_B}{x_B + K_{X, pred}} \right) \left( \frac{x_{pred}}{x_B} \right) \] (1)

2.3 Experiment (stage 2 and 3)

Results of the computer simulations have been compared with data from experiment in which activated sludge reactor was fed with mechanically treated wastewater. Parameters of experimental reactor and its conditions have been listed in table 3.

| Parameter                        | Value                        |
|----------------------------------|------------------------------|
| Sludge retention time [d]        | 28                           |
| Hydraulic retention time [d]     | 1.5                          |
| Temperature [°C]                 | 19                           |
| pH                               | 7.5                          |
| Dissolved oxygen concentration [gO2/m³] | mean value = 2, substantial variations occurred |
| Volume [ml]                      | 4100                         |

After steady state of reactor is acquired, subsequent measurements of nitrifiers mass were conducted (fig. 3.). Mass of nitrifiers were calculated on basis of AUR measurements, yield coefficients and growth rates from model. Calculated masses of AOB were used to calibrate protozoa influence.

![Fig. 3. AOB concentration and ammonia uptake rate during experiment.](image)

3 Results (stage 4)

Performed simulations show high sensitivity of a model for changes in values of protozoa kinetic parameters. Results presented on figure 4 display change of nitrifiers mass in a reactor in 11 days span presented on figure 3. Simulations have been run for 5 different values of
$k_{\text{pred}}$ parameter: 0.6; 0.8; 0.85 and 1. Best fitting index have been acquired for $k_{\text{pred}}=0.82$ and it equaled 2227.07.

![Graph showing mass of AOB in reactor for simulated and real data during experiment period.](image)

**Fig. 4.** Mass of AOB in reactor for simulated and real data during experiment period.

Substitute decay rate (equation 1) for aerobic conditions have been calculated for each variant and presented on figures 5 to 9. It can be noticed that simulations characterized as best fitted to experimental data are the ones in which values of substitute decay rate are closest to values of decay rates presented for models without excluding predation as separate process (classical values of decay rates for AOB are in range of 0.2 d$^{-1}$). Variations in values are due to changing oxygen concentrations.

![Graph showing substitute decay rate in aerobic conditions for $k_{\text{pred}}=1.00$.](image)

**Fig. 5.** Substitute decay rate in aerobic conditions for $k_{\text{pred}}=1.00$. 
It is worth noting that model is very sensitive to protozoa parameters. Mediocre increase in $k_{\text{pred}}$ (from 0.85 to 1.0) leads to dramatic increase in substitute decay rate (fig 10.) and complete elimination of AOB from reactor (fig. 4.). Mediocre decrease in $k_{\text{pred}}$ (from 0.85 to 0.6) leads to large increase of AOB mass (fig 4.).
4 Conclusions

Main conclusions are:

- Model with separate grazing can be properly calibrated to reflect mass of AOB obtained in laboratory experiment.
- Substitute decay rate is a tool that can be used to check whether calculated influence of grazing is in compliance with calibration results of classical models. It is shown that calibrated value is in accordance with classical ones.
- Model is very sensitive to parameters of protozoa. Minor increase in rate of predation \( k_{\text{pred}} \) leads to complete elimination of AOB while minor decrease leads to large overestimation of AOB population. This is important problem and will be addressed in further research.

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