Abstract: Massive and excessive use of nitrogen fertilizers and sustained irrigation have been widely practiced in recent years. This strategy leads to a large transfer of nitrates to groundwater, leading to a major environmental problem of nitrate contamination in water, intended for drinking water consumption. One of the most effective solutions is the degradation of nitrites and nitrates, into gaseous nitrogen, using the heterotrophic bacteria during the denitrification process. In this paper, we present and study a mathematical model of the biodenitrification process taking into account the fixed and mobile bacteria. This process is modeled by a system of ordinary differential equations and requires the success of bacteria to colonize the reactor. We study the existence and the asymptotic behaviour of the solution. We show the existence of a value of the injected carbon concentration from which we ensure the success of the biodenitrification process and we propose a heuristic algorithm which serves to control the biodenitrification process over time. Finally, we present some numerical simulations in to support the theoretical results.

Keywords: Bioprocesses, Biodenitrification, Ordinary differential equations, Asymptotic behavior

I. INTRODUCTION

Food safety and environmental protection are two key issues in nowadays societies. Due to a remarkable increase of the world population and in order to increase the yield of agricultural production, massive and excessive use of nitrogen fertilizers and sustained irrigation have been widely practised in recent years. This strategy leads to a large transfer of nitrates to groundwater, leading to a major environmental problem of nitrate contamination in water, intended for drinking water consumption. However, the natural environment can offer a possibility of remediation. One of the most effective solutions is the degradation of nitrites and nitrates, into gaseous nitrogen, is the use of heterotrophic bacteria during the denitrification process. This process is characterized by the fact that when the oxygen concentration is negligible, it will be replaced by oxidized nitrogen (NO2 and/or NO3) which is the best electron acceptor after oxygen so the biodenitrification process can be described as a respiratory process in which denitrifying bacteria use nitrates instead of oxygen as an acceptor of electrons [1–3]. It generally takes place under conditions, called anoxic, intended to provide energy for cell activity and the synthesis of new cells [4–6]. Successful biodenitrification process requires that the nitrate should be considered in the modeling as a limiting compound [7].

In [8], the authors studied the dynamics of potassium in the rhizosphere of the wheat root in the presence and in the absence of a biophysical source and they showed that the presence of bio-fertilizer leads to significant changes in potassium concentration in the rhizosphere of the wheat root. In [9], the authors proposed a
mathematical model to study the effect of water flux on nitrate dynamics in the rhizosphere of a single maize root taking into account important mechanisms such as diffusion and mass flux of solutes in the rhizosphere.

Charpentier et al. [10] modeled the Trichloroethylene (TCE) biodegradation process where they considered two types of bacteria, the first one degrades the contaminant while the second forms a biobarrier to prevent the contamination from infiltration into groundwater. They gave four spatialized models according to the consumption of the nutrient by the two types of bacteria. In [11] Charpentier et al. gave numerical simulations of several types of collaboration of two types of bacteria to control pollutants.

Recent theoretical and experimental studies on biofilms [12–14] confirm that the growth of bacteria on the walls can be a serious problem for bioreactors and fermenters and have fundamental implications for natural environments. But in biodegradation processes, colonization of the reactor by bacteria can be useful for the results obtained. Freter et al. [15,16] confirmed that adherent bacteria play an important role in the observed stability of the microflora of the mammalian large intestine to colonization by invading organisms.

Chatelier [17] conducted an experimental study for the assembly of an in situ biological denitrification unit, taking advantage of this self-purifying potential of the natural environment. By using an experimental device, he evaluated the effect of the formation of the biofilm on the surface of the aquifer, on the hydrodynamic properties of the initial medium. It also conjectured on the type of carbonaceous substrate to be used to ensure high denitrification yields. For biodegradation processes, adherent bacteria also play an important role for the evolution of bacteria in the reactor because free bacteria can be influenced by the flow rate.

As a generalization of Freter’s model, we find in [18–22] different models of bacterial attachment to walls, in the context of competition for the same substrate and a limited colonization site. F. Chevron [2], during the experiments, has confirmed the existence of free bacteria in the reactors which accelerated the biodenitrification. M. Ballyk et al. [18] analyzed the case of a single strain of bacteria for the general model given by Ballyk et al. [19] they have shown that there are two possible steady state regimes, the first is the total leaching of bacteria from the reactor (washout) and the second is the successful colonization of the reactor by the bacterial strain. They have shown that one of these regimes is stable under conditions related to system parameters.

In [7], the authors presented a model of the biodenitrification process taking into account the limitation of the kinetics by both the carbon source and the oxidized nitrogen, without studying the asymptotic behavior. This model concerns a single type of bacteria growing on nitrate, which splits into adherent bacteria or free bacteria in the liquid. The main objective of the present paper is to present a mathematical model of the biodenitrification process, taking into account both the fixed and mobile bacteria, and to give deeper insights and predictions on the evolution of bacteria and substrates involved, and consequently their exploitation in heuristic and/or optimal control algorithms.

The paper is organized as follows. In the next section, model and assumptions are presented and discussed. Then, the dynamics are mathematically analyzed in the following section. In particular, we show that there exists a value of the injected carbon concentration from which we ensure the success of the biodenitrification process. Finally, we perform some numerical simulations in agreement with the theoretical results and we propose a heuristic algorithm to control the biodenitrification process.

II. MATHEMATICAL MODEL

In order to describe the mathematical model that governs the water biodenitrification process, we consider that the reactor contains nitrified water, denitrifying bacteria and nutrients. The bacteria in the reactor are divided into two categories: a first type which adhere to the walls of the reactor with a maximum value denoted by $\omega_\infty$, forming a biofilm, denoted by $b_f$, and a second type which remain mobile and free floating, called planktonic cells and denoted by $b_m$.

The evolution of each type of bacteria is given by the following equation (see [23])

$$\frac{db_i(t)}{dt} = \left( \mu_i(S) - K_i \right) b_i(t), \quad \text{for } i = m, f, \quad (1)$$

where $b_i(t)$ and $S(t)$ represent respectively the concentrations of bacteria and substrate at time $t$. Symbols $K_i$ and $\mu_i$ are successively the loss coefficient (mortality, change of category, ...) and the specific growth rate which will be described later.

These denitrifying bacteria react with the contaminant nitrate, considered as an electron acceptor, its concentration will be denoted by $S_N$. Moreover, bacteria also need carbon for its growth, its concentration will be denoted by $S$. The input substrate concentrations of $S$ and $S_N$ are respectively $S^{\text{in}}$ and $S_N^{\text{in}}$, and we consider that denitrified water is extracted at the outlet

of the reactor. Based on the fundamental relations of biological kinetic (that are the growth rate of bacteria and the use rate of the substrate), the evolution of the concentrations \( S \) and \( S_N \), is governed respectively by the following equations [23]:

\[
\frac{dS(t)}{dt} = (S^m - S(t))d_S - \frac{1}{Y_m}b_m\mu_m(\cdot) - \frac{1}{Y_f}\gamma^{-1}b_f\mu_f(\cdot),
\]

(2)

\[
\frac{dS_N(t)}{dt} = (S^m_N - S_N(t))d_{S_N} - \frac{R}{Y_m}b_m\mu_m(\cdot) - \frac{R}{Y_f}\gamma^{-1}b_f\mu_f(\cdot),
\]

(3)

where \( Y_m \) and \( Y_f \) are successively the yield coefficient of mobile bacteria and adherent bacteria. Parameters \( R, d_S \) and \( d_{S_N} \) represent respectively the degradation rate of nitrates, the substrate dilution rate and the nitrate dilution rate. Symbol \( \gamma \) represents the conversion coefficient from volume density to areal density. It can be considered as the ratio between the surface and the perimeter of a section of the domain in the flow direction [18].

Generally, the evolution of the bacteria \( b_f \) and \( b_m \) depend on each other. In what follows, we describe and introduce this interdependence.

- **Case f.** We take firstly the equation (1) governing the fixed bacteria i.e. \( i = f \), and we consider that the loss coefficient \( K_f \) is the sum of both the mortality rate, denoted \( k_f \), and the term corresponding to the detachment rate of these bacteria from surfaces, denoted by \( \beta \), then we have

\[
K_f = k_f + \beta.
\]

(4)

In agreement with [15] and [16], only a proportion of the daughter cells of the fixed bacteria find place on the surface of the reactor, the rest of cells will be evacuated into the liquid.

Let us consider the variable \( \bar{b}_f \) defined by

\[
\bar{b}_f(t) := \frac{b_f(t)}{\omega(\cdot)}.
\]

(5)

The first proportion of daughter cells is a function of \( \bar{b}_f \) (see [15]), denoted by \( G(\bar{b}_f) \). In what follows we use the Freter’s formula (see [16]), given by

\[
G(X) = \frac{1 - X}{a + 1 - X},
\]

(6)

where \( a \) is a very small constant. Therefore, the growth rate of the fixed bacteria in the evolution equation (1) will be multiplied by the proportion \( G(b_f) \). Taking into account these improvements, from (1)-(6)-(4) we get

\[
\frac{db_f(t)}{dt} = \left( \mu_f(\cdot)G(\bar{b}_f) - k_f - \beta \right)b_f(t).
\]

(7)

In addition, a portion of planktonic bacteria can be attached to surfaces with a rate \( \alpha \). Therefore, the term \( \alpha b_m \) will be added to the equation (7) as source term. We consider \( (1 - \bar{b}_f) \) as the availability of the adhesion surface and we introduce \( \gamma \) the conversion coefficient of the volume density into a surface density. Taking into account these progress, the equation (7) becomes

\[
\frac{db_f(t)}{dt} = \left( \mu_f(\cdot)G(\bar{b}_f(t)) - k_f - \beta \right)b_f(t)
\]

\[
+ \alpha(1 - \bar{b}_f(t))\gamma b_m(t).
\]

(8)

- **Case m.** Moving to the mobile bacteria, similarly to the previous case, we consider that the loss coefficient \( K_m \) is the sum of both the mortality coefficient, denoted by \( k_m \), the dilution coefficient denoted by \( d \) and the attachment coefficient of mobile bacteria denoted by \( \alpha \) i.e. \( K_m = k_m + \alpha + d \). The evolution equation (1) of \( b_m \) becomes

\[
\frac{db_m(t)}{dt} = \left( \mu_m(\cdot) - k_m - \alpha - d \right)b_m(t).
\]

(9)

In addition, the following modelling improvements will be introduced to this equation. The source term corresponding to the surface concentration of the detached fixed bacteria, multiplied by the conversion coefficient, will be considered and denoted by \( \gamma^{-1}\beta b_f \). The concentration of bacteria which do not find a location to attach themselves will be taken into account and denoted by the term: \( b_f(t)\gamma^{-1}\mu_f(\cdot)(1 - G(b_f(t))) \). We obtain

\[
\frac{db_m(t)}{dt} = \left( \mu_m(\cdot) - k_m - \alpha - d \right)b_m(t)
\]

\[
+ \gamma^{-1}\beta b_f(t)\left( \mu_f(\cdot)(1 - G(\bar{b}_f(t))) + \beta \right).
\]

Finally, from the equations (8), (9), (2) and (3) we obtain the following system (10) describing the evolution of the quantities \( (b_f, b_m, S, S_N) \) of the
biodenitrification model:

\[
\begin{aligned}
\frac{db_f(t)}{dt} &= \left( \mu_f(\cdot)G(b_f(t)) - k_f - \beta \right) b_f(t) \\
&\quad + \alpha \gamma (1 - \bar{b}_f(t)) b_m(t), \\
\frac{db_m(t)}{dt} &= \left( \mu_m(\cdot) - k_m - d - \alpha (1 - \bar{b}_f(t)) \right) b_m(t) \\
&\quad + \left( \mu_f(\cdot) (1 - G(b_f(t))) + \beta \right) \gamma^{-1} b_f(t), \\
\frac{dS(t)}{dt} &= \left( S^\text{in} - S(t) \right) dS - \left( \frac{\mu_m(\cdot)}{Y_m} \right) b_m(t) \\
&\quad - \left( \frac{\mu_f(\cdot)}{Y_f} \gamma^{-1} \right) b_f(t), \\
\frac{dS_N(t)}{dt} &= \left( S_N^\text{in} - S_N(t) \right) dS_N - \left( \frac{R \mu_m(\cdot)}{Y_m} \right) b_m(t) \\
&\quad - \left( \frac{R \mu_f(\cdot)}{Y_f} \gamma^{-1} \right) b_f(t).
\end{aligned}
\]

At time \( t = 0 \), we assume that the reactor contains nitrified water with an initial density \( S_0^N \), mobile bacteria with an initial density \( b_0^f \), fixed bacteria with an initial density \( b_0^m \), and substrate with an initial density \( S_0 \):

\[
\begin{aligned}
b_f(0) &= b_0^f, \\
b_m(0) &= b_0^m, \\
S(0) &= S_0, \\
S_N(0) &= S_0^N.
\end{aligned}
\]

The system (10) becomes:

\[
\begin{aligned}
\frac{dc_1(t)}{dt} &= F_1(t, C(t)), \\
\frac{dc_2(t)}{dt} &= F_2(t, C(t)), \\
\frac{dc_3(t)}{dt} &= F_3(t, C(t)), \\
\frac{dc_4(t)}{dt} &= F_4(t, C(t)),
\end{aligned}
\]

where

\[
\begin{aligned}
F_1(t, C) &= \left( \mu_f(c_3, c_4)G\left( \frac{c_1}{\omega_{\infty}} \right) - k_f - \beta \right) c_1 \\
&\quad + \alpha \gamma \left( 1 - \frac{c_1}{\omega_{\infty}} \right) c_2, \\
F_2(t, C) &= \left( \mu_m(c_3, c_4) - k_m - d - \alpha \left( 1 - \frac{c_1}{\omega_{\infty}} \right) \right) c_2 \\
&\quad + \left( \mu_f(c_3, c_4) \left( 1 - G\left( \frac{c_1}{\omega_{\infty}} \right) \right) + \beta \right) \gamma^{-1} c_1, \\
F_3(t, C) &= \left( c_3^\text{in} - c_3 \right) dS - \left( \frac{\mu_m(c_3, c_4)}{Y_m} \right) c_2 \\
&\quad - \left( \frac{\mu_f(c_3, c_4)}{Y_f} \gamma^{-1} \right) c_1, \\
F_4(t, C) &= \left( c_4^\text{in} - c_4 \right) dS_N - \left( \frac{R \mu_m(c_3, c_4)}{Y_m} \right) c_2 \\
&\quad - \left( \frac{R \mu_f(c_3, c_4)}{Y_f} \gamma^{-1} \right) c_1.
\end{aligned}
\]

As we have mentioned before, \( \mu_f \) and \( \mu_m \) represent the growth function of adherent and free bacteria, respectively, and can obey the multiplicative formula (see [7])

\[
\mu_i(c_3, c_4) = \mu_{\text{max}}^i \left( \frac{c_3}{c_3 + K_1^i} \right) \left( \frac{c_4}{c_4 + K_2^i} \right), \quad i = f, m,
\]

where the indices \( i = m, f \) and \( j = 1, 2 \), ("\( j = 1 \)" relates to consumption of nutrient and "\( j = 2 \)" relates to consumption of nitrates) are in fact the Monod’s law constants, also called the half-saturation constants. See the profile of \( \mu_i \) in Figure 1, for example for \( \mu_{\text{max}}^f = 0.7, K_1^f = 60 \) and \( K_2^f = 50 \).
According to [7], the functions $G$ and $\mu_i(\cdot, \cdot)$ satisfy the following properties – Hypothesis H1 and H2:

**H1** The specific growth rate function of bacteria $\mu_i(x, y)$, for $i = f, m$, satisfies

$$\mu_i \in C^1, \quad \mu_i(0, y) = \mu_i(x, 0) = 0.$$  

**H2** The function $G$ is bounded such that

$$G \in C^1, \quad 0 < G(0) \leq 1, \quad G(1) = 0.$$  

### III. Asymptotic Behaviour Study

**Definition 1.** The function $F$ such that

$$F := (F_1, F_2, \ldots, F_p) \in C^1(\mathbb{R}_+ \times \mathbb{R}^p, \mathbb{R}^p)$$

with $p \in \mathbb{N}^*$ is quasi-positive if

$$F_i(t, y) \geq 0 \quad \forall (t, y) \in (0, +\infty) \times \mathbb{R}_+^p$$  

is such that $y_1 = 0$, where $y = (y_i)_{1 \leq i \leq p}$.

**Proposition 1.** Under Hypothesis H1 and H2 the system (12) admits a nonnegative maximal solution.

*Proof:* Under Hypothesis H1 and H2 we have $\mu_i \in C^1$ for $i = m, f$ and $G \in C^1$ then the functions $F_i$ are in $C^1$ for $i = 1, 2, 3, 4$ and so Lipschitz. Therefore, according to the Cauchy-Lipschitz theorem, the system (12) admits a maximal solution. In addition, the function $F = (F_1, F_2, F_3, F_4)$ is quasi-positive (i.e, for $c_i = 0$ for $c_j \geq 0$, $j = 1, 2, 3, 4$ we have $F_i(c_1, c_2, c_3, c_4) \geq 0$) and consequently the solutions are nonnegative (see [24], page 3).

**Proposition 2.** Let $(c_1, c_2, c_3, c_4)$ be the solution of (13), where $c_i^0 \leq c_i^{in}$ for $i = 3, 4$. We consider the following quantities

$$M_1 = \max \left( \frac{\mu_f(c_i^{in}, c_4^{in}) - k_f}{c_i^{in}}, \frac{\mu_m(c_3^{in}, c_4^{in}) - k_m - d}{c_3^{in}} \right),$$

$$M_2 = \min \left( \frac{\mu_f(c_3, c_4) - k_f}{c_3^{in}}, \frac{\mu_m(c_3, c_4) - k_m - d}{c_3^{in}} \right).$$

Thus,

- if $M_1 \leq 0$, then $\gamma^{-1} c_1(t) + c_2(t) \leq \gamma^{-1} c_1^0 + c_2^0$,
- if $M_2 \geq 0$, then $\gamma^{-1} c_1(t) + c_2(t) \geq \gamma^{-1} c_1^0 + c_2^0$.

*Proof:* Multiplying the first equation of the system (13) by $\gamma^{-1}$ and then making the sum of the first and the second equation of the system (13), we get

$$\frac{d(\gamma^{-1} c_1 + c_2)}{dt} = \gamma^{-1} \left( \mu_f(c_3, c_4) - k_f \right) c_1 + \left( \mu_m(c_3, c_4) - k_m - d \right) c_2.$$  

Or the functions $\mu_f$ and $\mu_m$ are increasing, for each variable, so

$$M_2 \left( \gamma^{-1} c_1 + c_2 \right) \leq \frac{d(\gamma^{-1} c_1 + c_2)}{dt} \leq M_1 \left( \gamma^{-1} c_1 + c_2 \right).$$

If $M_1 \leq 0$ then the function $(\gamma^{-1} c_1 + c_2)(t)$ decrease, from where

$$\forall t \geq t_0, \quad (\gamma^{-1} c_1 + c_2)(t) \leq \gamma^{-1} c_1^0 + c_2^0.$$  

If $M_2 \geq 0$ then the function $(\gamma^{-1} c_1 + c_2)(t)$ increase, from where

$$\forall t \geq t_0, \quad (\gamma^{-1} c_1 + c_2)(t) \geq \gamma^{-1} c_1^0 + c_2^0.$$

**Remark 1.**

- The first point of Proposition 2 confirms that the concentration of bacteria will be lower than the initial data and therefore the bacteria will die out and the biodenitrification process will be unsuccessful.
- The second point of Proposition 2 confirms that the concentration of bacteria will be greater than the initial data, and consequently the bacteria will succeed in colonizing the reactor and the biodenitrification process will be successful.

**Corollary 1.** Let $(c_1, c_2, c_3, c_4)$ be the solution of (13).

*Under the Hypothesis H1 and H2, for $i = 3$ or 4, we get

$$c_i(t) \leq c_i^{in}, \quad \forall t > 0.$$*
Proof: Thanks to Proposition 1, for \( i = 1 \) or 2 we have \( c_i \geq 0 \) then
\[
\begin{cases}
  \frac{dc_3}{dt} \leq (c_3^{in} - c_3) d_S, \\
  \frac{dc_4}{dt} \leq (c_4^{in} - c_4) d_{SN},
\end{cases}
\]
which implies
\[
\begin{cases}
  \frac{d(c_3 - c_3^{in})}{dt} \leq -(c_3 - c_3^{in}) d_S, \\
  \frac{d(c_4 - c_4^{in})}{dt} \leq -(c_4 - c_4^{in}) d_{SN},
\end{cases}
\]
equivalent to
\[
\begin{cases}
  \frac{d(c_3 - c_3^{in})}{dt} \leq (c_3 - c_3^{in})(-d_S), \\
  \frac{d(c_4 - c_4^{in})}{dt} \leq (c_4 - c_4^{in})(-d_{SN}).
\end{cases}
\]
By using the Gronwall’s lemma [25, Proposition 1.4], [26, Lemma 1], we get
\[
\begin{cases}
  c_3 - c_3^{in} \leq (c_0 - c_3^{in}) \exp(\lambda d t), \\
  c_4 - c_4^{in} \leq (c_0 - c_4^{in}) \exp(\lambda d_{SN} t),
\end{cases}
\]
and by the fact that \( c_0^1 \leq c_3^{in} \) and \( c_0^2 \leq c_4^{in} \) we obtain
\[
\begin{cases}
  c_3 - c_3^{in} \leq 0, \\
  c_4 - c_4^{in} \leq 0.
\end{cases}
\]
Finally we get
\[
\begin{cases}
  c_3 \leq c_3^{in}, \\
  c_4 \leq c_4^{in}.
\end{cases}
\]

Proposition 3. There exists a value \( c_3^* \) such that for any \( c_3^{in} \) verifying the inequality \( c_3^{in} < c_3^* \) we get \( M_1 < 0 \), where
\[
c_3^* = \min \left( \frac{(K_2^{in} + c_4^{in}) k_f (K_1^f + \mu_{f \max})}{c_4^{in} \mu_{\max}}, \frac{(K_2^{mm} + c_4^{in}) (k_m + d) (K_1^{in} + \mu_{m \max})}{c_4^{in} \mu_{\max}} \right).
\]
Proof: The conditions for that \( M_1 < 0 \) are
\[
\begin{align*}
\mu_f(c_3^{in}, c_4^{in}) - k_f &< 0, \\
\mu_m(c_3^{in}, c_4^{in}) - k_m - d &< 0.
\end{align*}
\]
According to (15), the first condition (17) implies
\[
\begin{align*}
\mu_{\max} & \frac{c_3^{in}}{K_1 + c_3^{in}} \times \frac{c_4^{in}}{K_2^f + c_4^{in}} - k_f < 0 \\
\Leftrightarrow & \frac{c_3^{in}}{K_1^f + \mu_{\max}^f} < \frac{(K_2^f + c_4^{in}) k_f}{c_4^{in} \mu_{\max}^f} \\
\Leftrightarrow & c_3^{in} < \frac{(K_2^f + c_4^{in}) k_f (K_1^f + \mu_{\max}^f)}{c_4^{in} \mu_{\max}^f}.
\end{align*}
\]
The second condition (18) implies
\[
\begin{align*}
\mu_{\max}^m & \frac{c_3^{in}}{K_1^{in} + c_3^{in}} \times \frac{c_4^{in}}{K_2^{mm} + c_4^{in} - k_m - d} < 0 \\
\Leftrightarrow & \frac{c_3^{in}}{K_1^{in} + \mu_{\max}^m} < \frac{(K_2^{mm} + c_4^{in}) (k_m + d)}{c_4^{in} \mu_{\max}^m} \\
\Leftrightarrow & c_3^{in} < \frac{(K_2^{mm} + c_4^{in}) (k_m + d) (K_1^{in} + \mu_{\max}^m)}{c_4^{in} \mu_{\max}^m}.
\end{align*}
\]
From (19) and (20), we obtain
\[
c_3^{in} < \min \left( \frac{(K_2^f + c_4^{in}) k_f (K_1^f + \mu_{f \max})}{c_4^{in} \mu_{\max}^f}, \frac{(K_2^{mm} + c_4^{in}) (k_m + d) (K_1^{in} + \mu_{m \max})}{c_4^{in} \mu_{\max}^m} \right) = c_3^*.
\]

Proposition 4. Let \( (c_1, c_2, c_3, c_4) \) be the solution of (13). The following properties hold true:
- If \( M_1 < 0 \), then
  \[
  \lim_{t \to \infty} (\gamma^{-1} c_1(t) + c_2(t)) = 0,
  \]
- If \( M_2 > 0 \), then
  \[
  \lim_{t \to \infty} (\gamma^{-1} c_1(t) + c_2(t)) = c^* > \gamma^{-1} c_1^0 + c_2^0.
  \]

Proof: By multiplying the first equation of (12) by \( \gamma^{-1} \) and making the sum of the first and the second equation of the system (12), we get
\[
\begin{align*}
\frac{d(\gamma^{-1} c_1 + c_2)}{dt} &= \gamma^{-1} \left( \mu_f(c_3, c_4) - k_f \right) c_1 \\
&\quad + \left( \mu_m(c_3, c_4) - k_m - d \right) c_2,
\end{align*}
\]
which implies
\[
M_2 \left( \gamma^{-1} c_1 + c_2 \right) \leq \frac{d(\gamma^{-1} c_1 + c_2)}{dt} \leq M_1 \left( \gamma^{-1} c_1 + c_2 \right).
\]
By using the Gronwall's lemma [26, Lemma 1], we have

\[ 0 \leq (\gamma^{-1}c_1 + c_2) \leq (\gamma^{-1}c_1^0 + c_2^0)e^{M_1 t}, \]

since \( M_1 < 0 \) and for \( t \to \infty \) then we obtain the result.

For the adherent bacteria and from (5), we have \( c_1 \leq \omega_\infty \). For the free bacteria and according to the growth function proprieties there exists a maximal concentration \( c_2^{max} \) such that \( c_2 \leq c_2^{max} \), which gives

\[ \gamma^{-1}c_1(t) + c_2(t) \leq \gamma^{-1}\omega_\infty + c_2^{max}. \]

Or \( M_2 > 0 \), then the concentration \( \gamma^{-1}c_1 + c_2 \) is increasing, respecting time, and bounded. Then there exists a value \( c^* \) such that

\[ \lim_{t \to \infty} (\gamma^{-1}c_1(t) + c_2(t)) = c^* > \gamma^{-1}c_1^0 + c_2^0. \]

According to Proposition 1, we have \( c_i \geq 0, i = 1, 2 \), then

\[ \begin{align*}
    \frac{dc_3}{dt} & \leq (c_3^{in} - c_3) d_s, \\
    \frac{dc_4}{dt} & \leq (c_4^{in} - c_4) d_{SN},
\end{align*} \]

which implies

\[ \begin{align*}
    \frac{d(c_3 - c_3^{in})}{dt} & \leq -(c_3 - c_3^{in}) d_s, \\
    \frac{d(c_4 - c_4^{in})}{dt} & \leq -(c_4 - c_4^{in}) d_{SN},
\end{align*} \]

equivalent to

\[ \begin{align*}
    \frac{d(c_3 - c_3^{in})}{dt} & \leq (c_3 - c_3^{in})(-d_s), \\
    \frac{d(c_4 - c_4^{in})}{dt} & \leq (c_4 - c_4^{in})(-d_{SN}).
\end{align*} \]

Using the Gronwall's lemma [26, Lemma 1], we get

\[ \begin{align*}
    c_3 - c_3^{in} & \leq (c_3^0 - c_3^{in}) \exp(-d_s t), \\
    c_4 - c_4^{in} & \leq (c_4^0 - c_4^{in}) \exp(-d_{SN} t),
\end{align*} \]

which implies

\[ \begin{align*}
    c_3 & \leq (c_3^0 - c_3^{in}) \exp(-d_s t) + c_3^0, \\
    c_4 & \leq (c_4^0 - c_4^{in}) \exp(-d_{SN} t) + c_4^{in}.
\end{align*} \]

Finally we obtain \( \lim_{t \to +\infty} c_3 \leq c_3^{in} \) and \( \lim_{t \to +\infty} c_4 \leq c_4^{in} \).

In what follows, we study the existence and stability of the steady state. By combining the first and the second equation of (12) in a single equation, the system (12) can be writing in the following form

\[ \begin{align*}
    \frac{d}{dt}(\gamma^{-1}c_1 + c_2) = \gamma^{-1} \left( \mu_f(c_3, c_4) - k_f \right) c_1 \\
    - \left( \mu_m(c_3, c_4) - k_m - d \right) c_2, \\
    \frac{dc_3}{dt} = (c_3^{in} - c_3) d_s - \left( \frac{\mu_m(c_3, c_4)}{Y_m} \right) c_2 \\
    - \left( \frac{\mu_f(c_3, c_4)}{Y_f} \right) \gamma^{-1} c_1, \\
    \frac{dc_4}{dt} = (c_4^{in} - c_4) d_{SN} - \left( \frac{R\mu_m(c_3, c_4)}{Y_m} \right) c_2 \\
    - \left( \frac{R\mu_f(c_3, c_4)}{Y_f} \right) \gamma^{-1} c_1.
\end{align*} \]

(21)

Define new variables \( \tilde{c}, \mu(c_3, c_4), K \). We make a change of variable

\[ \tilde{c} = \gamma^{-1}c_1 + c_2, \]

\[ \mu(c_3, c_4) \tilde{c} = \mu_f(c_3, c_4) \gamma^{-1} c_1 + \mu_m(c_3, c_4) c_2, \]

\[ K\tilde{c} = k_f \gamma^{-1} c_1 + (k_m + d) c_2, \]

\[ \frac{\mu(c_3, c_4)}{Y} \tilde{c} = \left( \frac{\mu_m(c_3, c_4)}{Y_m} \right) c_2 + \left( \frac{\mu_f(c_3, c_4)}{Y_f} \right) \gamma^{-1} c_1. \]

The system (21) then can be written as follows with three variables

\[ \begin{align*}
    \frac{d\tilde{c}}{dt} = (\mu(c_3, c_4) - K) \tilde{c}, \\
    \frac{dc_3}{dt} = (c_3^{in} - c_3) d_s - \frac{\mu(c_3, c_4)}{Y} \tilde{c}, \\
    \frac{dc_4}{dt} = (c_4^{in} - c_4) d_{SN} - \frac{R\mu(c_3, c_4)}{Y} \tilde{c}.
\end{align*} \]

(22)

The steady states, denoted by \( (\tilde{c}^*, c_3^*, c_4^*) \), of the system (22) verify

\[ \begin{align*}
    0 = (\mu(c_3^*, c_4^*) - K) \tilde{c}^*, \\
    0 = (c_3^{in} - c_3^*) d_s - \frac{\mu(c_3^*, c_4^*)}{Y} \tilde{c}^*, \\
    0 = (c_4^{in} - c_4^*) d_{SN} - \frac{R\mu(c_3^*, c_4^*)}{Y} \tilde{c}^*.
\end{align*} \]

(23)

We remark that the first steady state is the washout steady state and is \( (0, c_3^{in}, c_4^{in}) \). In addition, the other steady states satisfy

\[ \begin{align*}
    0 = (c_3^{in} - c_3^*) d_s - \frac{K}{Y} \tilde{c}^*, \\
    0 = (c_4^{in} - c_4^*) d_{SN} - \frac{RK}{Y} \tilde{c}^*.
\end{align*} \]

(24)
which implies
\begin{align}
0 &= (c^{in}_3 - c^*_3)d_S - \frac{K}{\gamma}c^*, \\
\dot{c}_4^* &= \frac{c^{in}_4 d_{SN} - R d_S (c^{in}_3 - c^*_3)}{d_{SN}},
\end{align}
(25)

hence
\begin{align}
\dot{c}^* &= \frac{Y d_S (c^{in}_3 - c^*_3)}{K}, \\
\dot{c}_4^* &= \frac{c^{in}_4 d_{SN} - R d_S (c^{in}_3 - c^*_3)}{d_{SN}}.
\end{align}
(26)

From the system (25), we remark that the system (23) admits an infinity of steady states. The linearization of the system (23) near the washout steady state is given using the following Jacobian matrix:
\[
A = \begin{pmatrix}
\mu_f(c^{in}_3, c^{in}_4) - K & 0 & 0 \\
-\mu_f(c^{in}_3, c^{in}_4) & -d_S & 0 \\
-\mu_f(c^{in}_3, c^{in}_4) & Y & 0 \\
& & -d_{SN}
\end{pmatrix}.
\]

**Proposition 5.** The following properties hold true:
- If $M_1 + M_2 < 0$, then the washout steady state is stable.
- If $M_1 + M_2 > 0$, the washout steady state is unstable and there is another state of equilibrium $(\dot{c}_1, \dot{c}_2, \dot{c}_3)$ such that $\dot{c}_1 \geq \gamma^{-1}c^0_1 + c^0_2$ and it is stable.

**Proof:**
- The condition $M_1 + M_2 < 0$ gives $\mu_f(c^{in}_3, c^{in}_4) - K < 0$ and then the matrix $A$ has three negative eigenvalues and therefore the washout steady state is stable.
- The condition $M_1 + M_2 > 0$ gives $\mu_f(c^{in}_3, c^{in}_4) - K > 0$ and then the matrix $A$ admits a non-negative eigenvalue and therefore the washout steady state is unstable. Using the Proposition 2 and the fact that the system (23) admits a solution then we obtain the result.

**Remark 2.** Another particular case can be obtained when $M_1 + M_2 = 0$, which gives $\mu_f(c^{in}_3, c^{in}_4) - K = 0$ and then the matrix $A$ admits 0 as an eigenvalue. Therefore, it is necessary to use another method for discussing this special case, such as the strategy carried out in [14].
IV. NUMERICAL RESULTS

In this section we present some numerical results about the success of the biodenitrification process in agreement with the theoretical results the problem (12). The different parameters used are given in Table II and come from [7]. From the same reference, we take the initial conditions of the system as indicated in Table I.

In the theoretical study, we showed the existence of a value $c_3^*$ of the injected carbon concentration from which we ensure the success of the biodenitrification process and the stability of the steady states. To verify that numerically, we perform two numerical experiences. Before that we start to compute the value of $c_3^*$. For example, with the parameter values given in the Table II and according to (16), we get $c_3^* = 0.6504mg/l$.

In the first test, we take a value of the amount of carbon injected $c_3^{in}$ such that $c_3^{in} \geq c_3^*$. For example with $c_3^{in} = c_3(0) = 104mg/l$, we get Figures 2, 3 and 4 (evolution/behaviour of bacteria and substrates). Figure 2 shows the evolution of $c_1$, $c_2$, $c_3$ and $c_4$. Figure 3 shows the evolution of $c$, $c_3$ and $c_4$ and then the stability of the system in agreement with Proposition 5. Figure 4 shows the evolution $(\gamma^{-1})c_1 + c_2$ and $(\gamma^{-1})c_1^0 + c_2^0$. We remark in this figure that for $c_3^{in} \geq c_3^*$, bacteria evolve in the reactor and then the success of the biodenitrification process which is consistent with the results obtained in the Proposition 2 and the Corollary 1. We also observe that from a certain time the four components stagnate which means that we obtain a stable steady state as is confirmed in the Propositions 4 and 5.

In the second test, we take a value of the amount of carbon injected $c_3^{in}$ such that $c_3^{in} \leq c_3^*$. For example with $c_3^{in} = c_3(0) = 0.5mg/l$, Figure 5 shows the evolution of $c_1$, $c_2$, $c_3$, $c_4$ and $c$, and then the stability of the system in agreement with Proposition 5. Figure 6 shows the evolution $(\gamma^{-1})c_1 + c_2$ and $(\gamma^{-1})c_1^0 + c_2^0$. We remark in this figure that for $c_3^{in} \leq c_3^*$, bacteria disappear from the reactor and then the bacterial concentration fails which is consistent with the results obtained in the Proposition 2 and the Corollary 1, we note that in this case we have $M_1 = -0.0011$, the value given in the Proposition 2.

We also observe that from a certain time bacteria concentration decreases towards 0 and the concentrations of carbon and nitrates, respectively, increase towards $c_3^{in}$ and $c_1^{in}$, which means that $(0,0,c_3^{in},c_1^{in})$ is a stable steady state as confirmed in the Propositions 4 and 5.

A. Numerical algorithm for solving the system

The functions of the second members of system (12) are given in the general form, by writing them as a function of $t$ and of the unknown vector $C(t) = (c_1(t), c_2(t), c_3(t), c_4(t))$. However, in our case, the system can be written as an autonomous system, i.e. $F_i$ are only functions of $C(t)$.

The numerical scheme for solving the problem (12) has been implemented in Python computational software through the fourth-order Runge-Kutta method (see Algorithm 1). It is an efficient method for solving problems of ordinary differential equations with initial values and it guarantees a stable computing time [27].

Algorithm 1 Numerical algorithm based on RK4 for solving the problem.

1: Initialisation: $t_0$ (initial time).
2: $t_f$ (final time),
3: $\Delta t$ (the step size),
4: $C \leftarrow C(t_0)$ (initial conditions).
5: define $F_i(C)$, $i = 1, 2, 3, 4$
6: $N \leftarrow \frac{t_f-t_0}{\Delta t} + 1$
7: $t \leftarrow t_0$
8: for $k \leftarrow 1$ to $N$ do
9: $K_1^i \leftarrow \Delta t F_i(C(t))$, $i = 1, 2, 3, 4$
10: $K_2^i \leftarrow \Delta t F_i\left(C(t) + \frac{\Delta t}{2} K_1^i\right)$, $i = 1, 2, 3, 4$
11: $K_3^i \leftarrow \Delta t F_i\left(C(t) + \frac{\Delta tK_2^i}{2}\right)$, $i = 1, 2, 3, 4$
12: $K_4^i \leftarrow \Delta t F_i\left(C(t) + \Delta t K_3^i\right)$, $i = 1, 2, 3, 4$
13: $c_i(t) \leftarrow c_i(t) + \frac{1}{6}(K_1^i + 2K_2^i + 2K_3^i + K_4^i)$, $i = 1, 2, 3, 4$
14: Registration.
15: $t \leftarrow t + \Delta t$
16: end for
B. Heuristic algorithm for improving the process

In the theoretical study of the problem as well as in the previous numerical tests, we showed the existence of a specific value of the injected carbon concentration from which we ensure the success of the biodenitrification process. In this part of numerical results, we propose a heuristic algorithm, based on previous results, to improve and control the biodenitrification process over time.

In what follow, we will present and compare 2 different schemes, to manage a total available quantity of $c_3$, to improve the process (one of them using our proposed heuristic algorithm Algorithm 2). We recall that $c_3$ is the nutrient that helps bacteria $c_2$ to increase, and thus the consumption of nitrates $c_4$ (contamination).

In both schemes, as already shown, we consider the correct given path that gives the success of the biodenitrification process, i.e. $c_3^{in} \geq c_3^*$, but in the heuristic algorithm scheme we make several injections of $c_3$ until the disappearance of the total available quantity of $c_3$ to manage, or until the elimination of $c_4$ (i.e. contamination). Improving biodenitrification involves reducing $c_4$ as much as possible. As we have shown, this is related to the value of $c_3$ available in the biodegradable medium. We assume that the total available quantity to manage is denoted by $c_3^{total} := c_3(0) + c_3^{in} + \sum_i c_3^{added,i}$, where $c_3^{added,i}$, $i = 1,2,\ldots$ are the quantities that will be added during different injections over time. In this experiment, we consider for example that $k_f = 0.05$ and $c_4^{in} = 50$ in order to have another value of $c_3^*$ larger than the one we obtained previously. So in this case, we get $c_3^* = 8.6714$ (see line 6 in Algorithm 2). In addition, we assume for example that $c_3^{total} = 21.025$.

As we have shown, $c_3^{in}$ must be greater than $c_3^*$ and $c_3^{(t_0)}$. Therefore, we start our heuristic algorithm by choosing $c_3^{in} = c_3^*$ (see line 8 in Algorithm 2). Then, we solve the system until the equilibrium or when $c_4$ begins to grow. The stopping time of this resolution is denoted $t^*$ (see line 10 in Algorithm 2). At this moment, we inject a quantity, denoted $c_3^{added,1}$ such that we obtain $c_3(t_1) + c_3^{in} = 2c^*$ (see line 13 in Algorithm 2). The same step will be done until the consumption of the total available quantity of $c_3$ to manage (or when $c_4 = 0$).

Figures 7 and 8 show the evolution of $c_4$ in the two schemes respectively, i.e without the use of the heuristic algorithm (scheme 1) and with the use of the heuristic algorithm (scheme 2), with the same total available quantity $c_3^{total}$.

#### Algorithm 2 Heuristic algorithm for improving the process.

1: **Input Parameters.**
2: **Initialisation:** $t_0 = 0$ (initial time),
3: $c_1(t_0), c_2(t_0), c_3(0), c_4(0)$,
4: $c_3^{total}, c_3^{in},$
5: $c_3^{total} := c_3(0) + c_3^{in} + \sum_i c_3^{added,i}$
6: $c_3^*$ by using (16) and $c_3^{in}$.
7: **We suppose that at $t_0$: $c_3(0) > c_3^*$
8: $c_3^{in} \leftarrow c_3(0)$
9: $t_i \leftarrow t_0$
10: (**#**): **Solve the System (10) on $[t_i, t^*]$**, were $t^*$ is the first moment to achieve equilibrium or when $c_4$ begins to grow.
11: $t_1 \leftarrow t^*$
12: $c_3^{added,1} \leftarrow c_3^* - c_3(t_1)$
13: $c_3(t_1) \leftarrow c_3(t_1) + c_3^{added,1}$
14: $t_i \leftarrow t_1$
15: **do (**#**).**
16: $t_2 \leftarrow t^*$
17: $c_3^{added,2} \leftarrow c_3^* - c_3(t_2)$
18: $c_3(t_2) \leftarrow c_3(t_2) + c_3^{added,2}$
19: $t_i \leftarrow t_2$
20: **do (**#**).**
21: **:**
22: until the consumption of the total quantity available of $c_3$ to manage (or when $c_4 = 0$).

For scheme 1 the resolution was made on $[0, 100]$ and thus Figure 7 shows the evolution of $c_4$ on $[0,100]$, devised over two intervals in order to subsequently show the compared curves.

However, with the heuristic algorithm 2, 3 steps were performed to manage the total available quantity $c_3^{total}$ (see Figure 8).

The first resolution was performed on $[t_0 = 0, t_1 = 39.9]$, and the quantity $c_3^{added,1} = 2.6212$ was injected (and at $t_1$: $c_4(t_1) = 47.7757$). The second resolution was performed on $[t_1, t_2 = 44.4]$, and the quantity $c_3^{added,2} = 0.7933$ was injected (and at $t_2$: $c_4(t_2) = 47.6265$). The third resolution was performed on $[t_2, t_3 = 45.8]$, and the quantity $c_3^{added,3} = 0.2677$ was injected (and at $t_3$: $c_4(t_3) = 47.6137$).

At instant $t_3$ all the quantity $c_3^{total}$ is consumed: $c_3(0) = 8.6714, c_3^{in} = 8.6714, c_3^{added,1} = 2.6212, c_3^{added,2} = 0.7933, c_3^{added,3} = 0.2677$, and $c_3^{total} := c_3(0) + c_3^{in} + \sum_i c_3^{added,i} = 21.025$. 
Mostafa Abaali, Salih Ouchtout, Modelling and analysis of the biodenitrification process

In terms of comparison: with scheme 1, we obtained at $t = 45.8 = t_3$: $c_4(t_3) = 47.8190 =: v_1$, and then it increases to the value 49.5331 at $t = 100$ (see Figure 7). However with the use of Algorithm 2, we obtained $c_4(t_3) = 47.6137 = v_2 < v_1$ and we can also follow our process of decreasing $c_4$ if the total quantity available $c_3^{\text{total}}$ to manage is greater. Moreover, with scheme 2, at time $t_3$, we have a quantity $c_2$ greater than that obtained in scheme 1: $c_2(t_3) = 1.0528$ “scheme 1” Vs $c_2(t_3) = 1.1248$ “scheme 2” ($c_2$ is the bacteria that helps to reduce nitrates $c_4$).

V. CONCLUSION

In this work we have developed a mathematical model dedicated to the biodenitrification process, taking into account the fixed and mobile bacteria. We studied asymptotic behaviour and provided some information for the solution of the problem. The numerical simulations in agreement with the theoretical results show the existence of a value of the injected carbon concentration from which we ensure the success of the biodenitrification process.

This study also allowed us to provide a heuristic algorithm in order to improve the situation of biodegradation by minimizing as much as possible the nitrate (contaminant) over time in the environment and then improving the biodenitrification process.

As a perspective, taking into account the obtained results, the model can be treated in a complete study of optimal control for choosing optimally the proportion of splitting and the corresponding re-introduction time(s) to obtain the best performances. This could be the matter of a future work.

Moreover, the introduction of spatial heterogeneity in the model, in terms of a system of reaction diffusion “partial differential equations” instead of a system of “ordinary differential equations” would be a reasonable extension of the present work, taking into consideration that spatial heterogeneity is usually observed in real problem.

REFERENCES

Mostafa Abaali, Salih Ouchtout, Modelling and analysis of the biodenitrification process


