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Dynamics of a chemostat with three competitive hydrogen oxidizing denitrifying microbial populations and their efficiency for denitrification

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ABSTRACT

In this work, competition for two nitrogen resources (nitrate-, nitrite-nitrogen) between three hydrogen oxidizing denitrifying populations (*Acidovorax* sp. strain Ic3 (X_1), *Paracoccus* sp. strain Ic1 (X_2), and *Acinetobacter* sp. strain Ic2 (X_3)) was examined. The dynamics of three systems of microbial populations (system I: $X_1 - X_3$, system II: $X_2 - X_3$, and system III: $X_1 - X_2 - X_3$), grown in a chemostat, was studied using bifurcation analysis. The chemostat is the most common type of biological reactor used for the study of microbial growth under controlled conditions. The effect of the operating parameters (i.e., dilution rate and feed nitrate nitrogen concentration) on the long-term behavior of the systems showed that X_3 was the predominant population for a wide range of combinations of dilution rate and feed nitrate nitrogen concentration. Also, coexistence of two populations (X_2X_3, X_1X_3) was observed. The results of the bifurcation analysis were also used to determine the denitrification rate and the nitrite nitrogen accumulation for each of the three systems as a function of the dilution rate (up to 0.17 h^{-1}) and the feed nitrate nitrogen concentration (up to 300 mg/L). The highest denitrification rate was achieved by system I (28 mg/Lh). A comparison between the three systems showed that the nitrite nitrogen concentration in system I was less than the one in system III, while the two systems gave similar denitrification rates. The second system had the greatest accumulation of nitrites with the lowest denitrification rate.

1. Introduction

An increasing interest in mixed cultures of organisms has been observed during the last three decades. Mixed cultures are more versatile than pure cultures, present higher yields and mediate some multistep chemical transformations. Since natural ecosystems consist mainly from mixed culture, detailed study of the behavior of these systems can improve our understanding of many phenomena (Fredrickson, 1983).

The various forms of nitrogen (ammonium-, nitrite- and nitrate-nitrogen) in natural ecosystems give rise to severe environmental problems, including eutrophication, deterioration of water quality and potential hazards to human health. Thus nitrogen removal-denitrification from water and wastewater has become mandatory in recent years (Marazioti et al., 2003). The World Health Organization has set the maximum level of nitrate in drinking water at 11.3 mg NO_3^--N/L and a limit on nitrite at 0.03 mg NO_2^--N/L (Council Directive 98/83/EC). Physical–chemical processes for nitrogen removal result in high operating and capital cost. Biochemical engineering offers biological denitrification as an attractive alternative for nitrate removal (Kapoor and Viraraghavan, 1997). Better understanding of the dynamic behavior of mixed culture systems will be an important guide in developing design and operating strategies for denitrification systems.

Hydrogenotrophic denitrification of drinking water has been extensively studied in our previous studies in batch assays (Vasiliadou et al., 2006a,b). Hydrogen can be used as alternative electron donor for autotrophic denitrification because of its clean nature, low biomass yield and relatively low cost. In addition, hydrogen does not persist in the treated water to create biological instability and no further steps are required to remove either excess substrate or its derivatives. Experiments were initially performed using a mixed culture originating from a wastewater treatment plant. A kinetic model was developed and its kinetic parameters were determined from the batch experiments (Vasiliadou et al., 2006a). In the second paper, three strains of the mixed culture were isolated (*Acidovorax* sp. strain Ic3, *Acinetobacter* sp. strain Ic2, *Paracoccus* sp. strain Ic1) and batch growth of each population was studied in pure culture, under anoxic hydrogenotrophic conditions in a defined synthetic medium (Vasiliadou et al., 2006b).

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Nomenclature

D	chemostat dilution rate (h^{-1})
k_{d1ad}	constant in growth rate expression of *Acidovorax* sp. strain Ic3 (mg NO_2^--N/mg NO_3^--N)
k_{d1an}	constant in growth rate expression of *Acinetobacter* sp. strain Ic2 (mg NO_2^--N/mg NO_3^--N)
k_{d1pr}	constant in growth rate expression of *Paracoccus* sp. strain Ic1 (mg NO_2^--N/mg NO_3^--N)
k_{d2ad}	constant in growth rate expression of *Acidovorax* sp. strain Ic3 (mg NO_3^--N/mg NO_2^--N)
k_{d2an}	constant in growth rate expression of *Acinetobacter* sp. strain Ic2 (mg NO_3^--N/mg NO_2^--N)
k_{d2pr}	constant in growth rate expression of *Paracoccus* sp. strain Ic1 (mg NO_3^--N/mg NO_2^--N)
k_{dad}	death rate constant of *Acidovorax* sp. strain Ic3 (h^{-1})
k_{dan}	death rate constant of *Acinetobacter* sp. strain Ic2 (h^{-1})
k_{dpr}	death rate constant of *Paracoccus* sp. strain Ic1 (h^{-1})
K_{iad}	nitrate inhibition constant of *Acidovorax* sp. strain Ic3 (mg/L)
K_{ian}	nitrate inhibition constant of *Acinetobacter* sp. strain Ic2 (mg/L)
K_{ipr}	nitrate inhibition constant of *Paracoccus* sp. strain Ic1 (mg/L)
K_{man}	nitrite inhibition constant of *Acinetobacter* sp. strain Ic2 (mg/L)
K_{mad}	nitrite inhibition constant of *Acidovorax* sp. strain Ic3 (mg/L)
K_{mpr}	nitrite inhibition constant of *Paracoccus* sp. strain Ic1 (mg/L)
K_{nad}	saturation constant for nitrite of *Acidovorax* sp. strain Ic3 (mg/L)
K_{nan}	saturation constant for nitrite of *Acinetobacter* sp. strain Ic2 (mg/L)
K_{npr}	saturation constant for nitrite of *Paracoccus* sp. strain Ic1 (mg/L)
K_{sad}	saturation constant for nitrate of *Acidovorax* sp. strain Ic3 (mg/L)
K_{san}	saturation constant for nitrate of *Acinetobacter* sp. strain Ic2 (mg/L)
K_{spr}	saturation constant for nitrate of *Paracoccus* sp. strain Ic1 (mg/L)
m_{1ad}	specific maintenance rate for *Acidovorax* sp. strain Ic3 (mg NO_3^--N/h mg biomass)
m_{1an}	specific maintenance rate for *Acinetobacter* sp. strain Ic2 (mg NO_3^--N/h mg biomass)
m_{1pr}	specific maintenance rate for *Paracoccus* sp. strain Ic1 (mg NO_3^--N/h mg biomass)
m_{2ad}	specific maintenance rate for *Acidovorax* sp. strain Ic3 (mg NO_2^--N/h mg biomass)
m_{2an}	specific maintenance rate for *Acinetobacter* sp. strain Ic2 (mg NO_2^--N/h mg biomass)
m_{2pr}	specific maintenance rate for *Paracoccus* sp. strain Ic1 (mg NO_2^--N/h mg biomass)
N_1	nitrate nitrogen concentrations (mg/L)
N_{1f}	nitrate nitrogen concentrations in the feed (mg/L)
N_2	nitrite nitrogen concentrations (mg/L)
r_{max1ad}	maximum specific growth rate on nitrate of *Acidovorax* sp. strain Ic3 (h^{-1})
r_{max1an}	maximum specific growth rate on nitrate of *Acinetobacter* sp. strain Ic2 (h^{-1})
r_{max1pr}	maximum specific growth rate on nitrate of *Paracoccus* sp. strain Ic1 (h^{-1})
r_{max2ad}	maximum specific growth rate on nitrite of *Acidovorax* sp. strain Ic3 (h^{-1})
r_{max2an}	maximum specific growth rate on nitrite of *Acinetobacter* sp. strain Ic2 (h^{-1})
r_{max2pr}	maximum specific growth rate on nitrite of *Paracoccus* sp. strain Ic1 (h^{-1})
X_1	cell mass concentration of *Acidovorax* sp. strain Ic3 (mg/L)
X_3	cell mass concentration of *Acinetobacter* sp. strain Ic2 (mg/L)
X_2	cell mass concentration of *Paracoccus* sp. strain Ic1 (mg/L)
Y_{sad}	growth yield coefficient on nitrate of *Acidovorax* sp. strain Ic3 (mg biomass/mg NO_3^--N)
Y_{san}	growth yield coefficient on nitrate of *Acinetobacter* sp. strain Ic2 (mg biomass/mg NO_3^--N)
Y_{spr}	growth yield coefficient on nitrate of *Paracoccus* sp. strain Ic1 (mg biomass/mg NO_3^--N)
Y_{nad}	growth yield coefficient on nitrite of *Acidovorax* sp. strain Ic3 (mg biomass/mg NO_2^--N)
Y_{nan}	growth yield coefficient on nitrite of *Acinetobacter* sp. strain Ic2 (mg biomass/mg NO_2^--N)
Y_{npr}	growth yield coefficient on nitrite of *Paracoccus* sp. strain Ic1 (mg biomass/mg NO_2^--N)

Mathematical models were developed to describe the kinetics of growth of the bacteria (pure cultures) and the values of the model parameters were determined through fitting to experimental data. Kinetics of bacterial growth was found to depend on both nitrate and nitrite, as substitutable nutrients, and be inhibited by them at high concentration values. Specific maintenance energy terms for nitrate and nitrite reduction were also included in the anoxic models. The mathematical models were able to describe accurately cell growth and nitrate and nitrite utilization measured experimentally.

The behavior and the denitrifying capability of the three bacteria was also studied in well-defined mixed batch cultures of the two species *Acidovorax* sp. strain Ic3–*Acinetobacter* sp. strain Ic2 (system I), the two species *Acinetobacter* sp. strain Ic2–*Paracoccus* sp. strain Ic1 (system II), and all three species *Acidovorax* sp. strain Ic3–*Acinetobacter* sp. strain Ic2–*Paracoccus* sp. strain Ic1 (system III). Based on the kinetic models that were developed to describe growth of each species in pure culture, mathematical models for the mixed cultures were developed and tested against data from the experimental system (Vasiliadou et al., 2006b).

In systems of mixed microbial populations, the most common microbial interaction appears to be competition, which is the situation in which two or more populations use at least one common resource and the resource has a dynamic effect on at least one of them. When it is the only interaction present, competition is called pure. We say that we have total competition when the populations compete for all the resources (nitrate-, nitrite nitrogen) that affect the growth rate of at least one of the populations (Fredrickson and Stephanopoulos, 1981). Biological reactors are used for the propagation of microbial populations. One common type of biological reactor is the chemostat, which is a well-stirred continuous reactor into which sterile liquid medium is fed at a constant rate (Novick and Szilard, 1950). This type of reactor is often used in water and wastewater treatment of various industrial processes.

In this work we study pure and total competition for two nitrogen resources among two or three hydrogen oxidizing denitrifying populations in a bioreactor by analysis of the corresponding mathematical models. Bifurcation analysis was used, in order to determine the influence of the operating parameters on the survival and