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Fate of commensalistic cultures in identical coupled bioreactors

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ABSTRACT

A comprehensive analysis of static and dynamic behavior of a mixed culture in two identical coupled bioreactors is presented considering anaerobic digestion involving acidogens (X) and methanogens (Y) as the example bioprocess. A single continuous culture may operate at up to seven steady states, including up to four coexistence steady states, with only one coexistence steady state being locally stable. The one-way interaction between X and Y allows for compartmentalization of the system for a stand-alone bioreactor and two coupled bioreactors into two subsystems, which facilitates the analysis of steady state types and stability characteristics of these and classification of dynamic behavior. The bioreactors in the two-reactor system are identical only in terms of feed composition and reactor space time. A two-reactor system may admit up to forty nine steady states, which are comprised of up to forty coexistence steady states, at least at very low interaction rate (R). The static and dynamic analysis of the two-reactor system is facilitated by appropriate grouping of large number of steady states arising for very low R into nine clusters. Numerical illustrations reveal the rich steady state structure of the bioprocess in coupled bioreactors. While a single bioreactor can operate at only one locally stable coexistence steady state, the coupled bioreactors can operate at up to five locally stable coexistence steady states over certain ranges of R. The two-reactor system is operationally more flexible and more robust vis-a-vis single reactor as concerns maintenance of mixed culture. Emergence of four additional steady state clusters and additional coexistence and partial washout steady states at intermediate R reveals that the coupled bioreactors are an example of a complex system.

1. Introduction

Observed in their natural environment, most living species are found to exist in organized social settings reflecting various degrees of intermingling with other living species. The prospects of survival and coexistence of these species in these environments is strongly impacted by the nature of the interactions between them and the type of environment they populate. Rarely is any species found to live in the total isolation, as mixed populations of organisms are obviously the rule rather than the exception in natural systems. The ecosystem at large and any of its subsections (of whatever size, e.g., lakes, ponds, and rivers of different sizes, seas, and oceans) are characterized by substantial spatial and temporal variations in these key variables and other such variables. These variations are responsible in part for the preservation of biodiversity. Humans have tried to mimic the functioning of living species in natural environments in "controlled" settings in research laboratories and industrial complexes. Even in these settings, spatial and temporal variations in key variables influencing the functioning of living species are increasingly common (Chang and Baltzis, 1989; Birol et al., 2002). Environmental partitioning in (bio)reactors in these controlled settings arises when multiple reactors are used in series-parallel configuration or when multichamber reactors (Shain, 1997) are used.

Microbial populations comprising two or more different organisms and inhabiting a common environment are known to interact in a number of different ways (Fredrickson and Tsuchiya, 1977; Shuler and Kargi, 2002). Mixed cultures involving wild-type and recombinant cell species are used extensively in commercial fermentations for production of a variety of food products, alcoholic beverages, and pharmaceuticals, biological waste treatment, and biological leaching, and are omnipresent in ecological systems (Parulekar and Lim, 1986a,b; Zeikus and Johnson, 1991).

Commensalism is an interaction in which one cell population (organism) is positively affected by the presence of the other, while the second population is not affected substantially by the presence of the first population (see Parulekar and Lim, 1986a,b; Shuler and Kargi, 2002; Zeikus and Johnson, 1991 for citations on experimental and theoretical studies). The theoretical studies on commensalistic cultures have focused on CSTR operation and have dealt with steady state characteristics (multiplicity and local stability) (Parulekar and Lim, 1986a,b; Parulekar and Ingle, 2006; Sheintuch, 1980; Stephanopoulos, 1981) and dynamic behavior, including admissibility and stability of cyclic states (Parulekar and Lim,

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1986a,b; Parulekar and Ingle, 2006). Two examples of mixed cultures exhibiting commensalism with competition are: (i) cultures containing cells genetically engineered to synthesize a target metabolite and cells these revert to upon loss of DNA vector responsible for synthesis of the target metabolite and (ii) cultures of healthy cells and cancerous cells in animal tissue and blood (Birol et al., 2002). The emergence phenomena to be discussed later in this work have been demonstrated for the first example by periodic need-based addition of antibiotic (Lee and Parulekar, 1996).

The simplest reactor system with partitioned environment is two coupled well-mixed reactors (CSTRs). This system can be viewed as an aggregation of two subsystems, the individual CSTRs, and displays the phenomenon of emergence featured by complexity theory. Emergence, in this sense, refers to any situation in which a system displays a level of functionality that is not possible for any of its subsystems when considered on their own. Behavioral patterns of two coupled chemical reactors have been studied substantially, with the focus being on the study of chaotic dynamics, quasiperiodicity, and resonance phenomena resulting from the coupling of oscillators and synchronization of these (Abashar and Judd, 1998; Chen et al., 1996; Taylor and Kevrekidis, 1993). When the interaction between living species in a mixed culture is competition, the ability to support coexistence of these species is lacking from the homogeneous single reactor system (Fredrickson and Tsuchiya, 1977), but emerges as a generic capability of the coupled reactor system with two-way exchange of cell culture between reactors (Baltzis and Fredrickson, 1983; Birol et al., 2002; Kung and Baltzis, 1987; Pavlou et al., 1990; Stephanopoulos and Fredrickson, 1979).

In the present work, steady state and dynamic behavior of continuous mixed cultures of two cell species with commensalistic interaction and kinetic feedback in coupled bioreactors, identical in terms of feed composition and reactor space time, is analyzed and investigated. Anaerobic digestion is considered as the example bioprocess. Background on the mechanism, kinetics, and modeling of anaerobic digestion is available in Parulekar and Ingle (2006). After problem formulation in Section 2, relevant background on single reactor operation, which is essential for analysis of two-reactor system, is provided in Section 3, with further details being available in Parulekar and Ingle (2006). Steady state analysis for two coupled reactors is provided in Section 4, including (i) local stability analysis, (ii) classification, multiplicity and stability of steady states for very weak interaction between the two reactors, and (iii) variations in steady states with variation in interaction. Illustrations on variations in steady state clusters and individual steady states and *emergence* of additional steady state clusters and additional steady states with increasing interaction are provided in Section 5. A qualitative discussion of dynamics of two-reactor system follows in Section 6.

2. Problem formulation

A modified version of Hill and Barth's model (Hill and Barth, 1977; Noykova et al., 2002), which provides a concise three-stage representation of anaerobic digestion incorporating mixed consortia of microorganisms, is adopted here. This process, involving acid producing bacteria (*acidogens*, X) and methane producing bacteria (*methanogens*, Y), can be represented as

$$A \xrightarrow{E} B \xrightarrow{X} C \xrightarrow{Y} P. \tag{1}$$

The feed to each bioreactor is composed of complex insoluble organics, A, which are hydrolyzed by extracellular enzymes, E, to simple soluble organics, B (Fig. 1). The densities of various streams for a single reactor or a two-reactor system are considered to be the same and the mass flow rates in and out of each reactor are considered to be equal. The extracellular enzymes are synthesized and secreted by the acidogens. The acidogens utilize B for their growth and generate volatile acids, C. The methanogens utilize C for their growth and generate, among other products, biogas, P, which is composed largely of methane and carbon dioxide.

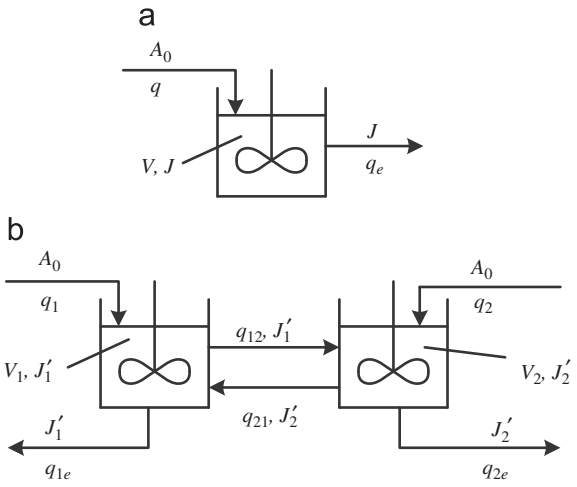

Fig. 1. Schematics of (a) a single well-mixed reactor and (b) two coupled well-mixed reactors. J=A, B, C, X, Y.

The first step in Eq. (1) involves a feedback effect of X (E synthesized by X), adding to the complexity of the process. Such kinetic feedback also occurs in other cell cultures, two notable examples being conversion of cellulose and starch by suitable cell species to target metabolites. Utilization of these substrates requires synthesis of extracellular enzymes, cellulases in the case of cellulose and amylases in the case of starch, by living cells. Together, cellulose and starch are the most abundant organic matter on this planet. The extracellular enzymes catalyze hydrolysis of cellulose and starch to glucose, which can be metabolized by living cells to allow for their growth and production of target metabolites.

For a stand-alone or a single CSTR [Fig. 1(a)], the conservation equations for the species in Eq. (1) influencing the kinetics are (Noykova et al., 2002; Parulekar and Ingle, 2006)

$$\frac{dA}{dt} = f_1, \quad f_1 = D(A_0 - A) - r_1, \tag{2}$$

$$\frac{dX}{dt} = f_2, \quad f_2 = -DX + (\mu_1 - k_1)X, \tag{3}$$

$$\frac{dB}{dt} = f_3, \quad f_3 = -DB + r_1 - \beta\mu_1 X, \tag{4}$$

$$\frac{dY}{dt} = f_4, \quad f_4 = -DY + (\mu_2 - k_2)Y, \tag{5}$$

$$\frac{dC}{dt} = f_5, \quad f_5 = -DC + \gamma\mu_1 X - \delta\mu_2 Y, \tag{6}$$

with the biogas production rate, Q, being expressed as

$$Q = \varepsilon\mu_2 Y. \tag{7}$$

The expressions for r_1, μ_1 and μ_2 are (Noykova et al., 2002):

$$r_1 = \alpha AX, \quad \mu_1 = \frac{\mu_{10}B}{(K_1 + B)}, \quad \mu_2 = \frac{\mu_{20}C}{(K_2 + C)(1 + C/K_I)}. \tag{8}$$

The values of kinetic parameters in Eqs. (2)–(8) are listed in Table 1 (Noykova et al., 2002; Simeonov and Stoyanov, 2003).