

## Article

# Mathematical Analysis of a Coupled Anaerobic Digestion and In Situ Biomethanation Model for Methane Production

Iliyass Ahlamine <sup>1,\*</sup> , Hamza Berga <sup>2</sup> , Abdellah Alla <sup>1</sup>  and Noha El Khattabi <sup>1,\*</sup> 

<sup>1</sup> LAMA Laboratory, Department of Mathematics, Faculty of Sciences, Mohammed V University in Rabat, Rabat 10000, Morocco; a.alla@um5r.ac.ma

<sup>2</sup> Interdisciplinary Research Laboratory in Innovation, Technology, and Management, Higher Institute of Management, Administration, and Computer Engineering (ISMAGI), Rabat 10120, Morocco; hamza.berga@ismagi.ac.ma

\* Correspondence: iliyass.ahlamine@um5r.ac.ma (I.A.); noha.elkhattabi@fsr.um5.ac.ma (N.E.K.)

## Abstract

In the context of renewable energy production, the anaerobic biodegradation of organic matter represents an efficient tool for methane production. However, the resulting biogas contains about 60% methane and 40% carbon dioxide, which limits its energetic value. To increase methane production, we study a coupled mathematical model that combines a three-stage anaerobic digestion process with hydrogenotrophic biomethanation and gas–liquid mass transfer. The coupled system is qualitatively analyzed, showing that the coupled model can admit twelve equilibria, and their local stability is subsequently investigated. Numerical simulations are performed to construct operating diagrams that identify the conditions leading to favorable methane production and to separate them from regions where microbial washout or bistability may occur. The results show that methane production is enhanced by suitable choices of the organic substrate, as well as acetate and hydrogen inflow concentrations, depending on the associated growth functions and the dilution rate. In bistable regions, high methane production can still be obtained under favorable initial concentrations.

**Keywords:** methane production; three-stage anaerobic digestion; hydrogenotrophic biomethanation; stability analysis; operating conditions; bistability

## 1. Introduction

Anaerobic digestion (AD) in bioreactors is a biological process that occurs in the absence of oxygen, in which organic matter is biodegraded and converted to biogas, mainly composed of methane and carbon dioxide [1].

In the literature, several mathematical models have been proposed to describe the AD process. Anaerobic digestion model No 1 (ADM1) [2] provides a detailed representation of the four biological stages (hydrolysis, acidogenesis, acetogenesis and methanogenesis) through a large system of nonlinear differential equations. In the context of wastewater treatment, Bernard et al. [3] introduced the simplified (AM2) model, which describes acidogenesis using the Monod growth function and methanogenesis using the Haldane growth function. This function accounts for the inhibition of methanogenesis in the presence of high acetate concentrations and indirectly reflects the effects of VFA accumulation, pH decrease and alkalinity reduction on the reactor's stability. Other modeling approaches have also been developed based on mass balance principles, resulting in simplified or detailed dynamic models of AD. A recent review classified these models according to



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their level of complexity, ranging from simple models to more detailed systems [4]. In the specific context of landfill bioreactors, the model introduced by Halvadakis [5] is of particular interest for the present study. This model focuses on three main biological stages (hydrolysis, acidogenesis, and methanogenesis) and employs the Monod bacterial growth function for both acidogenesis and methanogenesis.

In the context of mathematical analysis of continuous AD models, several studies have investigated the qualitative behavior of reduced order systems. Fekih-Salem et al. [6] analyzed a three-stage continuous model and established results concerning the existence and stability of equilibria. Benyahia et al. [7] studied a two-stage model and performed a bifurcation analysis. More recently, Hanaki et al. [8] considered a two-stage continuous system and characterized the reactor performance through operating diagrams, describing the relation between the inflow substrate concentrations and the dilution rate. Following this approach, Sari [9] used these operating diagrams to optimize the biogas production in one-step and AM2 models, considering the dilution rate and the influent substrate concentrations as operating parameters.

Biogas produced through AD is generally used for electricity and heat generation. To increase its energy value, carbon dioxide can be removed from methane. Another strategy, which is the subject of increasing research, is to convert the carbon dioxide to methane through biological methanation, using the injection of hydrogen. This conversion is carried out by the hydrogenotrophic methanobacterium biomass [10]. Biological methanation can be carried out in two main configurations. In the case of in situ biomethanation, the hydrogen is injected directly into the anaerobic digester, where the AD processes and hydrogenotrophic methanogenesis occur simultaneously. In contrast, ex situ biomethanation is carried out in a separate reactor specifically designed to optimize the activity of hydrogenotrophic methanogens [11]. Recent studies have examined both configurations in detail. ex situ biomethanation has been studied using dynamic and spatial modeling approaches, as in [12,13]. In parallel, in situ biomethanation has been explored within integrated AD systems, as in [10,14–16]. These works highlight the crucial role of coupling AD with hydrogenotrophic methanogenesis to increase methane production.

Many mathematical models have been proposed to describe the coupling between the anaerobic digestion, biomethanation, and gas–liquid mass transfer processes. First, Schill et al. [17] developed a continuous model for hydrogenotrophic methanogenesis coupled with gas–liquid mass transfer, and determined the equilibrium expressions. Recent studies on in situ biomethanation have shown the importance of the hydrogen supply, gas–liquid mass transfer and operating conditions for methane production. Mazzeo et al. [14] proposed a reduced model for an in situ biomethanation process, including organic matter degradation,  $H_2$ ,  $CO_2$  and  $CH_4$  dynamics, and gas–liquid transfer. Their results showed that hydrogen mass transfer is a limiting step of the process. Lembo et al. [10] showed that biogas recirculation improves hydrogen utilization and methane enhancement. From a mathematical perspective, the qualitative analysis of such coupled systems remains relatively limited. Borisov et al. [18] studied a reduced coupled model and examined the stability of the equilibria using numerical approaches. Other studies, such as those by Giovannini et al. [19] and Mudzanani et al. [20], have focused on the model formulation and parameter sensitivity.

In the present study, we perform a mathematical analysis of a model obtained by coupling the Halvadakis AD framework with in situ biomethanation and gas–liquid mass transfer effects, following the formulation proposed by Mazzeo et al. [14]. The model is based on the assumption that the culture medium is perfectly mixed and that the environmental conditions, in particular temperature and pH, remain within the appropriate operating ranges. In this model, the dissolved organic substrate is transformed by acido-

genic bacteria, whose growth is described by a Monod growth function. The produced volatile fatty acids (VFAs) are represented here by acetate, which is consumed by the methanogenic biomass. This methanogenic step is modeled by a Haldane growth function, allowing the inhibitory effect of the accumulation of acetate to be taken into account. We demonstrate that the solution of the dynamical system is positive and bounded, characterize the equilibria and their existence conditions, and study their local stability. Numerical simulations are used to construct operating diagrams with respect to the dilution rate and the inflow concentrations of organic substrate, acetate, and hydrogen. These diagrams provide a practical tool for identifying operating regions that favor methane production and separating them from regions associated with microbial washout or bistability. In bistable regions, high methane production can still be achieved, but the final state depends on the initial microbial concentrations. Therefore, the basins of attraction are also examined to identify suitable initial conditions leading to favorable methane production.

## 2. Materials and Methods

### 2.1. Three-Step Anaerobic Digestion

First, we introduce the mathematical model for the AD, inspired by the Halvadakis model [5]. The biodegradable organic substrate is hydrolyzed into dissolved organic substrate at a rate of  $k_h S_s$ . For a reactor with continuous liquid inflow and outflow, the equation for  $S_s$  includes the dilution term  $D(S_s^{\text{in}} - S_s)$ , where  $D$  is the dilution rate.

The dissolved substrate is consumed by the acidogenic biomass following the specific growth rate  $\mu_a(S_{dc})$ . The total substrate consumption rate is given by  $\frac{1}{y_a} \mu_a(S_{dc}) X_a$ , where  $y_a$  denotes the yield coefficient of acidogenic biomass. The fraction  $\mu_a(S_{dc}) X_a$  contributes to biomass growth, while the remaining fraction  $\frac{1 - y_a}{y_a} \mu_a(S_{dc}) X_a$  is converted into acetate and carbon dioxide. This fraction is partially converted to acetate with a yield  $y_{ac}$ , and the remaining portion contributes to the production of carbon dioxide.

The acetate substrate is then consumed by the methanogenic biomass at a specific growth rate  $\mu_m(S_{ac})$ . The total acetate uptake rate is  $\frac{1}{y_m} \mu_m(S_{ac}) X_m$ , where  $y_m$  is the methanogenic yield coefficient. The fraction  $\mu_m(S_{ac}) X_m$  supports the growth of methanogenic biomass, while the remaining portion  $\frac{1 - y_m}{y_m} \mu_m(S_{ac}) X_m$  is converted into carbon dioxide and methane, with respective yields  $y_{CO_2}$  and  $(1 - y_{CO_2})$ . The resulting products are considered as dissolved species in the liquid phase and are coupled with the gas phase through mass transfer processes.

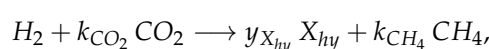
Finally, the two biomass populations are  $X_a$  and  $X_m$ , whose dynamics are described by

$$\frac{dX_a}{dt} = (\mu_a(S_{dc}) - D)X_a, \quad \frac{dX_m}{dt} = (\mu_m(S_{ac}) - D)X_m.$$

### 2.2. Hydrogenotrophic Methanogenesis and Gas–Liquid Coupling

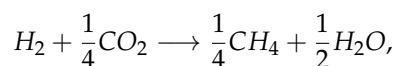
Hydrogenotrophic methanogenesis is described by two coupled mechanisms: a growth reaction and a maintenance reaction [14,17].

The growth reaction is written in the following stoichiometric form:



where  $y_{X_{hy}}$  is the yield coefficient of hydrogenotrophic methanobacterium biomass, while  $k_{CO_2}$  and  $k_{CH_4}$  denote the stoichiometric coefficients associated with carbon dioxide consumption and methane production, respectively.

In addition, the following maintenance reaction is taken into account:



which accounts for substrate consumption not related to growth.

The total volumetric hydrogen uptake rate is defined by  $r_{H_2} = \mu_{hy}(H_2) X_{hy}$ , where  $\mu_{hy}(H_2)$  is the specific hydrogen uptake rate. The maintenance consumption is modeled by  $v_M = mX_{hy}$ , with  $m$  being the maintenance coefficient. The growth hydrogen uptake is therefore  $v_G = r_{H_2} - v_M$ .

Hence, the rates of dissolved carbon dioxide consumption and methane production are given by

$$r_{CO_2} = k_{CO_2} v_G + \frac{1}{4}v_M, \quad r_{CH_4} = k_{CH_4} v_G + \frac{1}{4}v_M.$$

The reactor operates continuously in the liquid phase with a dilution rate  $D$ . Therefore, the dynamic of the hydrogenotrophic biomass is described by

$$\frac{dX_{hy}}{dt} = y_{X_{hy}}(\mu_{hy}(H_2) - m)X_{hy} - DX_{hy}.$$

Dissolved hydrogen, carbon dioxide and methane are coupled to the gas phase through Henry's law. The equilibrium concentrations  $H_2^*$ ,  $CO_2^*$  and  $CH_4^*$  satisfy

$$H_2^* = \frac{p_{H_2}}{h_{H_2}}, \quad CO_2^* = \frac{p_{CO_2}}{h_{CO_2}}, \quad CH_4^* = \frac{p_{CH_4}}{h_{CH_4}},$$

where  $p_{H_2}$ ,  $p_{CO_2}$  and  $p_{CH_4}$  are the partial pressures and  $h_{H_2}$ ,  $h_{CO_2}$  and  $h_{CH_4}$  denote Henry's constants. The gas–liquid mass transfer is modeled by the standard form

$$-k_{L,i} \left( i - \frac{p_i}{h_i} \right), \quad i \in \{H_2, CO_2, CH_4\},$$

which drive dissolved concentrations toward thermodynamic equilibrium with the corresponding partial pressures.  $k_{L,i}$  denote the mass transfer coefficient between the liquid and the gas phases for each species  $i$ .

### 2.3. Gas-Phase Pressure Dynamics

The evolution of  $p_{H_2}$ ,  $p_{CO_2}$ , and  $p_{CH_4}$  is subject to the gas–liquid mass transfer. The liquid phase is modeled as a continuous reactor with dilution rate  $D$ , while the gas phase is represented by a closed headspace without direct gas inflow or outflow to the external environment. Thus, the pressure dynamics only account for the gas–liquid mass transfer. This assumption is inspired by continuous liquid-phase configurations with a closed headspace, where gas purging is avoided in order to reduce the gaseous hydrogen losses to the exterior [21]. In the present model, this idea is applied to the hydrogenotrophic biomethanation, where the dissolved hydrogen participates in the conversion of carbon dioxide to methane. Here, this does not imply that the hydrogen cannot become limiting. It only means that direct gas-phase losses are neglected, while the hydrogen can still be transferred to the liquid phase, be consumed biologically, or leave the system in its dissolved form with the liquid outflow. The boundedness results proven in the next sections for the pressure dynamics indicate that under the assumptions of the model, the accumulation of gas in the closed headspace remains limited and does not become infinite. This result motivates further investigation of suitable pressure-control strategies for a continuous liquid-phase biomethanation model with a closed headspace.

The pressure dynamics are therefore written as

$$\frac{dp_i}{dt} = \frac{v_l}{v_g} r T k_{L,i} \left( i - \frac{p_i}{h_i} \right), \quad i \in \{H_2, CO_2, CH_4\},$$

where  $v_l$  and  $v_g$  denote the liquid and gas volumes,  $r$  is the universal gas constant, and  $T$  is the absolute temperature.

These equations are obtained by combining Henry's law with the ideal gas law, assuming a constant temperature and constant gas volume.

#### 2.4. Limiting Substrate Assumption

In this study, the hydrogenotrophic uptake rate is described by a Monod function depending on the dissolved hydrogen concentration:

$$\mu_{hy}(H_2) = q^{\max} \frac{H_2}{K_{H_2} + H_2},$$

where  $q^{\max}$  denotes the maximum uptake rate and  $K_{H_2}$  is the half-saturation constant for hydrogen. This formulation is used in biomethanation models when hydrogen is considered as the limiting substrate [14,17,22].

Hydrogenotrophic microorganisms consume both dissolved hydrogen and dissolved carbon dioxide. However, in the AD process and in situ biomethanation systems, carbon dioxide is produced by the digestion process and is more soluble than hydrogen. Consequently, when dissolved carbon dioxide remains sufficiently available, the dissolved hydrogen can be considered the limiting substrate, and the uptake rate can be expressed as a Monod function of  $H_2$  alone.

If the carbon dioxide becomes limiting, a double-substrate expression may be used, such as in the following formulation proposed by Inkeri et al. [23]:

$$\mu_{hy}(H_2, CO_2) = q^{\max} \frac{H_2}{K_{H_2} + H_2} \frac{CO_2}{10^{-3} + CO_2}.$$

#### 2.5. Mathematical Model

The mathematical model of the process described in Figure 1 is formulated as a system of ordinary differential equations. This system is represented as follows:

$$\begin{cases} \frac{dS_s}{dt} = -k_h S_s + D(S_s^{\text{in}} - S_s), \\ \frac{dS_{dc}}{dt} = k_h S_s - \frac{1}{y_a} \mu_a(S_{dc}) X_a + D(S_{dc}^{\text{in}} - S_{dc}), \\ \frac{dX_a}{dt} = (\mu_a(S_{dc}) - D) X_a, \\ \frac{dS_{ac}}{dt} = y_{ac} \left( \frac{1 - y_a}{y_a} \mu_a(S_{dc}) \right) X_a - \frac{1}{y_m} \mu_m(S_{ac}) X_m + D(S_{ac}^{\text{in}} - S_{ac}), \\ \frac{dX_m}{dt} = (\mu_m(S_{ac}) - D) X_m. \end{cases} \quad (1)$$

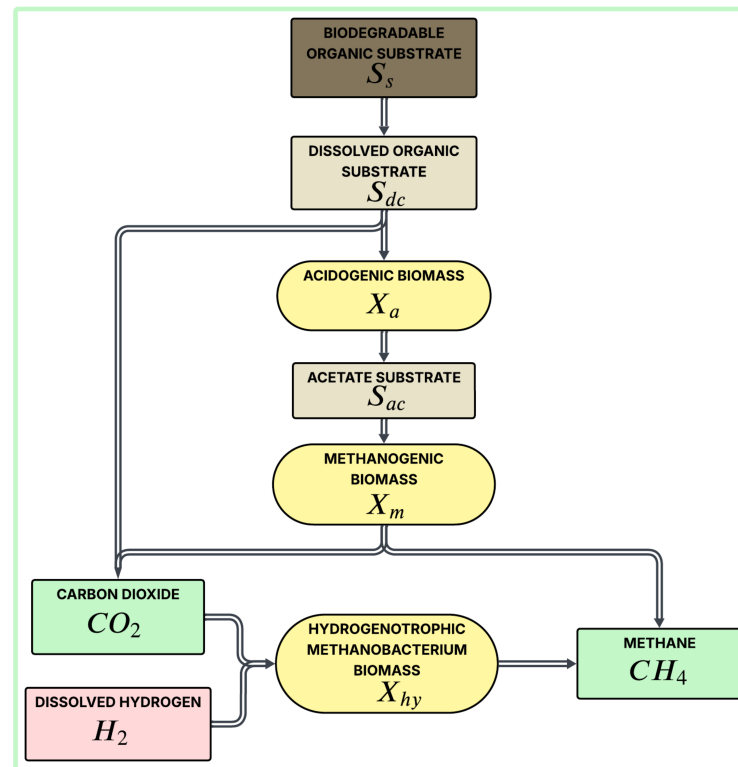


Figure 1. Schematic representation of the full process.

$$\begin{cases} \frac{dH_2}{dt} = D(H_2^{\text{in}} - H_2) - \mu_{hy}(H_2) X_{hy} - k_{L,H_2} \left( H_2 - \frac{p_{H_2}}{h_{H_2}} \right), \\ \frac{dX_{hy}}{dt} = (y_{X_{hy}}(\mu_{hy}(H_2) - m) - D) X_{hy}, \\ \frac{dp_{H_2}}{dt} = \frac{v_1}{v_g} rT \cdot k_{L,H_2} \left( H_2 - \frac{p_{H_2}}{h_{H_2}} \right). \end{cases} \quad (2)$$

$$\begin{cases} \frac{dCO_2}{dt} = D(CO_2^{\text{in}} - CO_2) + (1 - y_{ac}) \left( \frac{1 - y_a}{y_a} \mu_a(S_{dc}) \right) X_a + y_{CO_2} \left( \frac{1 - y_m}{y_m} \mu_m(S_{ac}) \right) X_m \\ \quad - k_{CO_2} (\mu_{hy}(H_2) - m) X_{hy} - \frac{1}{4} m X_{hy} - k_{L,CO_2} \left( CO_2 - \frac{p_{CO_2}}{h_{CO_2}} \right), \\ \frac{dp_{CO_2}}{dt} = \frac{v_1}{v_g} rT \cdot k_{L,CO_2} \left( CO_2 - \frac{p_{CO_2}}{h_{CO_2}} \right), \\ \frac{dCH_4}{dt} = -DCH_4 + (1 - y_{CO_2}) \left( \frac{1 - y_m}{y_m} \mu_m(S_{ac}) \right) X_m \\ \quad + k_{CH_4} (\mu_{hy}(H_2) - m) X_{hy} + \frac{1}{4} m X_{hy} - k_{L,CH_4} \left( CH_4 - \frac{p_{CH_4}}{h_{CH_4}} \right), \\ \frac{dp_{CH_4}}{dt} = \frac{v_1}{v_g} rT \cdot k_{L,CH_4} \left( CH_4 - \frac{p_{CH_4}}{h_{CH_4}} \right). \end{cases} \quad (3)$$

The initial conditions are denoted by

$$S_s(0) = S_s^0, \quad S_{dc}(0) = S_{dc}^0, \quad X_a(0) = X_a^0, \quad S_{ac}(0) = S_{ac}^0, \quad X_m(0) = X_m^0,$$

$$H_2(0) = H_2^0, \quad X_{hy}(0) = X_{hy}^0, \quad p_{H_2}(0) = p_{H_2}^0, \\ CO_2(0) = CO_2^0, \quad CH_4(0) = CH_4^0, \quad p_{CO_2}(0) = p_{CO_2}^0, \quad p_{CH_4}(0) = p_{CH_4}^0.$$

The bacterial growth functions  $\mu_a$  and  $\mu_m$  represent the growth rates of acidogenic and methanogenic biomass. In previous studies and practical scenarios, bacterial growth rates are modeled using Monod or Haldane functions. The Haldane growth function accounts for substrate inhibition effects, making it a more realistic choice for systems with high substrate concentrations, where high levels can inhibit the bacterial growth. This is the case for the acetate substrate concentration  $S_{ac}$ , namely

$$\mu_m(S_{ac}) = \frac{\mu_m^{max} S_{ac}}{K_a + S_{ac} + \frac{S_{ac}^2}{K_{ia}}},$$

where  $K_a$  and  $K_{ia}$  are, respectively, the half-saturation and the inhibition constants of the acetate substrate.

However, the Monod function is well suited for simpler scenarios with lower substrate concentrations and negligible inhibition effects. This is the case for dissolved organic and hydrogen substrates, whose concentrations in the reactor remain relatively low. Thus,

$$\mu_a(S_{dc}) = \frac{\mu_a^{max} S_{dc}}{K_d + S_{dc}} \quad \text{and} \quad \mu_{hy}(H_2) = q^{max} \frac{H_2}{K_{H_2} + H_2},$$

where  $K_d$  and  $K_{H_2}$  are the constants of half-saturation of the dissolved organic and dissolved hydrogen substrates, respectively.

**Variables.** The model variables are:

- $S_s$ : Total concentration of biodegradable organic substrate in the liquid phase ( $\text{g L}^{-1}$ );
- $S_{dc}$ : Dissolved organic substrate concentration ( $\text{g L}^{-1}$ );
- $X_a$ : Acidogenic biomass concentration ( $\text{g L}^{-1}$ );
- $S_{ac}$ : Acetate concentration ( $\text{g L}^{-1}$ );
- $X_m$ : Methanogenic biomass concentration ( $\text{g L}^{-1}$ );
- $H_2$ : Dissolved hydrogen concentration ( $\text{mol m}^{-3}$ );
- $p_{H_2}$ : Partial pressure of hydrogen (Pa);
- $X_{hy}$ : Hydrogenotrophic methanobacterium biomass concentration ( $\text{g L}^{-1}$ );
- $CO_2$ : Dissolved carbon dioxide concentration ( $\text{mol m}^{-3}$ );
- $p_{CO_2}$ : Partial pressure of carbon dioxide (Pa);
- $CH_4$ : Dissolved methane concentration ( $\text{mol m}^{-3}$ );
- $p_{CH_4}$ : Partial pressure of methane (Pa).

**Hypotheses:** The model parameters satisfy the following hypotheses:

**Hypothesis 1.** All constant parameters are non-negative:

$$D > 0, \quad k_h > 0, \quad K_a > 0, \quad K_{ia} > 0, \quad K_d > 0, \quad K_{H_2} > 0, \quad m > 0, \\ v_l > 0, \quad v_g > 0, \quad r > 0, \quad T > 0, \quad h_i > 0, \quad k_{L,i} > 0 \quad (i \in \{H_2, CO_2, CH_4\}),$$

and the input concentrations satisfy

$$S_s^{in} \geq 0, \quad S_{dc}^{in} \geq 0, \quad S_{ac}^{in} \geq 0, \quad H_2^{in} \geq 0, \quad CO_2^{in} \geq 0.$$

**Hypothesis 2.** The yield parameters satisfy

$$0 < y_a < 1, \quad 0 < y_m < 1, \quad 0 < y_{ac} < 1, \quad 0 < y_{CO_2} < 1, \quad 0 < y_{X_{hy}} < 1,$$

**Hypothesis 3.** We assume that the dilution rate is strictly smaller than the maximal specific growth rates:

$$0 < D < \mu_a^{\max}, \quad 0 < D < \mu_m^{\max}.$$

For the hydrogenotrophic population, we assume that

$$0 < a < q^{\max}, \quad \text{with} \quad a = \frac{D}{y_{X_{hy}}} + m.$$

**Hypothesis 4.** For all non-negative values of the state variables, we assume that

$$DCO_2^{\text{in}} + (1 - y_{ac}) \left( \frac{1 - y_a}{y_a} \mu_a(S_{dc}) \right) X_a + y_{CO_2} \left( \frac{1 - y_m}{y_m} \mu_m(S_{ac}) \right) X_m + k_{L,CO_2} \frac{p_{CO_2}}{h_{CO_2}} \geq k_{CO_2} (\mu_{hy}(H_2) - m) X_{hy} + \frac{1}{4} m X_{hy}.$$

The assumption (H4) specifies the condition under which the dissolved carbon dioxide remains sufficiently available, so that the hydrogenotrophic uptake rate can be written as a function of  $H_2$  only. The left-hand side of (H4) represents the total contribution of the dissolved carbon dioxide. This contribution comes from the influent, from the anaerobic digestion process, and from the transfer of carbon dioxide from the gas phase to the liquid phase. The right-hand side represents the carbon dioxide demand of the hydrogenotrophic bacteria. Thus, (H4) states that the available  $CO_2$  contribution is sufficient to cover this demand.

If the AD pathway and the gas–liquid transfer do not provide enough dissolved carbon dioxide, the increase in  $CO_2^{\text{in}}$  may help maintain its availability.

If  $CO_2$  becomes limiting, the hydrogenotrophic uptake rate cannot be described as a function of  $H_2$  alone, and an expression depending on both  $H_2$  and  $CO_2$  may be considered [23]. Thus, the assumption (H4) would be reduced.

### 3. Results

#### 3.1. Study of Asymptotic Behavior

As our System (1)–(3) satisfies the conditions of the Cauchy–Lipschitz theorem, the existence and uniqueness of the solution with a given initial value is guaranteed. We begin with a result on the non-negativity and boundedness of the solution of System (1)–(3).

##### 3.1.1. Positivity and Boundedness of the Solution

**Proposition 1.** Under hypotheses (H1–H3), for any non-negative initial condition, the solution of System (1)–(3) is non-negative for all  $t \geq 0$ .

**Proof.** The proof is given in Appendix A.1.  $\square$

**Proposition 2.** Under hypotheses (H1–H3), for any non-negative initial condition, the solution of System (1)–(3) is bounded for all  $t \geq 0$ .

**Proof.** The proof is given in Appendix A.2.  $\square$

##### 3.1.2. Equilibria of Subsystem (1)

Consider Subsystem (1) with the variable  $u = (S_s, S_{dc}, X_a, S_{ac}, X_m)$ . Define

$$S_s^* = \frac{D}{k_h + D} S_s^{\text{in}}, \quad S_{dc}^* = \frac{k_h S_s^* + D S_{dc}^{\text{in}}}{D}. \quad (4)$$

Let  $\lambda_a$  denote  $\lambda_a(D)$ , the unique solution of  $\mu_a(S_{dc}) = D$  (for the Monod function). For the Haldane function, let  $\lambda_m^- < \lambda_m^+$  denote  $\lambda_m^-(D) < \lambda_m^+(D)$ , where the two solutions of  $\mu_m(S_{ac}) = D$ .

Set

$$X_a^* = y_a(S_{dc}^* - \lambda_a), \quad S_{ac}^* = S_{ac}^{\text{in}} + y_{ac} \frac{1 - y_a}{y_a} X_a^*, \quad (5)$$

and, for  $\sigma \in \{-, +\}$ ,

$$X_m^{*\sigma} = y_m(S_{ac}^{\text{in}} - \lambda_m^\sigma), \quad X_m^{**\sigma} = y_m(S_{ac}^* - \lambda_m^\sigma). \quad (6)$$

**Proposition 3.** Under hypotheses (H1–H3), Subsystem (1) admits six non-negative equilibria:

$$\begin{aligned} E_1 &= (S_s^*, S_{dc}^*, 0, S_{ac}^{\text{in}}, 0); \\ E_2 &= (S_s^*, \lambda_a, X_a^*, S_{ac}^*, 0); \\ E_3 &= (S_s^*, S_{dc}^*, 0, \lambda_m^-, X_m^{*-}); \quad E_4 = (S_s^*, S_{dc}^*, 0, \lambda_m^+, X_m^{*+}); \\ E_5 &= (S_s^*, \lambda_a, X_a^*, \lambda_m^-, X_m^{*-}); \quad E_6 = (S_s^*, \lambda_a, X_a^*, \lambda_m^+, X_m^{*+}). \end{aligned}$$

Moreover,  $E_1$  always exists.  $E_2$  exists whenever  $X_a^* \geq 0$ .  $E_3$  (resp.  $E_4$ ) exists whenever  $X_m^{*-} \geq 0$  (resp.  $X_m^{*+} \geq 0$ ).  $E_5$  (resp.  $E_6$ ) exists whenever  $X_a^* \geq 0$  and  $X_m^{*-} \geq 0$  (resp.  $X_m^{*+} \geq 0$ ).

### 3.1.3. Jacobian Matrix of Subsystem (1)

Let  $u = (S_s, S_{dc}, X_a, S_{ac}, X_m)$  and denote  $\mu_a = \mu_a(S_{dc})$ ,  $\mu_m = \mu_m(S_{ac})$ ,  $\mu'_a = \mu'_a(S_{dc})$  and  $\mu'_m = \mu'_m(S_{ac})$ . The Jacobian matrix of Subsystem (1) at  $u$  is given by

$$J(u) = \begin{pmatrix} -(k_h + D) & 0 & 0 & 0 & 0 \\ k_h & -D - \frac{1}{y_a} \mu'_a X_a & -\frac{1}{y_a} \mu_a & 0 & 0 \\ 0 & \mu'_a X_a & \mu_a - D & 0 & 0 \\ 0 & y_{ac} \frac{1 - y_a}{y_a} \mu'_a X_a & y_{ac} \frac{1 - y_a}{y_a} \mu_a & -D - \frac{1}{y_m} \mu'_m X_m & -\frac{1}{y_m} \mu_m \\ 0 & 0 & 0 & \mu'_m X_m & \mu_m - D \end{pmatrix}. \quad (7)$$

It follows that  $\lambda_1 = -(k_h + D)$  is an immediate eigenvalue of  $J(u)$ . Moreover, the remaining eigenvalues are those of the two  $2 \times 2$  blocks

$$A = \begin{pmatrix} -D - \frac{1}{y_a} \mu'_a X_a & -\frac{1}{y_a} \mu_a \\ \mu'_a X_a & \mu_a - D \end{pmatrix}, \quad B = \begin{pmatrix} -D - \frac{1}{y_m} \mu'_m X_m & -\frac{1}{y_m} \mu_m \\ \mu'_m X_m & \mu_m - D \end{pmatrix}. \quad (8)$$

**Lemma 1.** Let  $u = (S_s, S_{dc}, X_a, S_{ac}, X_m)$  and consider the blocks  $A$  and  $B$  defined in (8).

- **Block A.** If  $X_a = 0$ , then  $A$  is upper-triangular and its eigenvalues are

$$\lambda_2 = -D, \quad \lambda_3 = \mu_a(S_{dc}) - D. \quad (9)$$

If  $X_a \neq 0$ , the eigenvalues of  $A$  are the solutions of

$$\lambda^2 - \text{tr}(A) \lambda + \det(A) = 0, \quad (10)$$

where

$$\text{tr}(A) = \mu_a(S_{dc}) - 2D - \frac{1}{y_a} \mu'_a(S_{dc}) X_a, \quad \det(A) = D \left( D - \mu_a(S_{dc}) + \frac{1}{y_a} \mu'_a(S_{dc}) X_a \right). \quad (11)$$

- **Block B.** If  $X_m = 0$ , then  $B$  is upper-triangular and its eigenvalues are

$$\lambda_4 = -D, \quad \lambda_5 = \mu_m(S_{ac}) - D. \quad (12)$$

If  $X_m \neq 0$ , the eigenvalues of  $B$  are the solutions of

$$\lambda^2 - \text{tr}(B)\lambda + \det(B) = 0, \quad (13)$$

where

$$\text{tr}(B) = \mu_m(S_{ac}) - 2D - \frac{1}{y_m} \mu'_m(S_{ac}) X_m, \quad \det(B) = D \left( D - \mu_m(S_{ac}) + \frac{1}{y_m} \mu'_m(S_{ac}) X_m \right). \quad (14)$$

**Proposition 4.** Assume that the equilibrium  $E_5 = (S_s^*, \lambda_a, X_a^*, \lambda_m^-, X_m^{*-})$  exists. Then,  $E_5$  is locally asymptotically stable.

**Proof.** The proof is given in Appendix A.3.  $\square$

**Proposition 5.** Assume that the equilibria  $E_4 = (S_s^*, S_{dc}^*, 0, \lambda_m^+, X_m^{*+})$  and  $E_6 = (S_s^*, \lambda_a, X_a^*, \lambda_m^+, X_m^{*+})$  exist. Then, both  $E_4$  and  $E_6$  are unstable.

**Proof.** The proof is given in Appendix A.4.  $\square$

**Proposition 6.** Assume that the equilibria  $E_1, E_2, E_3$  exist. Then:

1.  $E_1 = (S_s^*, S_{dc}^*, 0, S_{ac}^{\text{in}}, 0)$  is locally asymptotically stable if and only if  $\mu_a(S_{dc}^*) < D$  and  $\mu_m(S_{ac}^{\text{in}}) < D$ .
2.  $E_2 = (S_s^*, \lambda_a, X_a^*, S_{ac}^*, 0)$  is locally asymptotically stable if and only if  $\mu_m(S_{ac}^*) < D$ .
3.  $E_3 = (S_s^*, S_{dc}^*, 0, \lambda_m^-, X_m^{*-})$  is locally asymptotically stable if and only if  $\mu_a(S_{dc}^*) < D$ .

**Proof.** The proof is given in Appendix A.5.  $\square$

Table 1 summarizes the existence and the local stability conditions of the equilibria of Subsystem (1).

**Table 1.** Existence and local stability of the equilibria of Subsystem (1). S: Stable; U: unstable.

| Equilibrium | Existence Conditions                                          | Local Stability                                                                                            |
|-------------|---------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| $E_1$       | always                                                        | $S_{dc}^* < \lambda_a(D)$ , $S_{ac}^{\text{in}} < \lambda_m^-(D)$ or $S_{ac}^{\text{in}} > \lambda_m^+(D)$ |
| $E_2$       | $S_{dc}^* \geq \lambda_a(D)$                                  | $S_{ac}^* < \lambda_m^-(D)$ or $S_{ac}^* > \lambda_m^+(D)$                                                 |
| $E_3$       | $S_{ac}^{\text{in}} \geq \lambda_m^-(D)$                      | $S_{dc}^* < \lambda_a(D)$                                                                                  |
| $E_4$       | $S_{ac}^{\text{in}} \geq \lambda_m^+(D)$                      | U                                                                                                          |
| $E_5$       | $S_{dc}^* \geq \lambda_a(D)$ , $S_{ac}^* \geq \lambda_m^-(D)$ | S                                                                                                          |
| $E_6$       | $S_{dc}^* \geq \lambda_a(D)$ , $S_{ac}^* \geq \lambda_m^+(D)$ | U                                                                                                          |

### 3.1.4. Equilibria of Subsystem (2)

Consider Subsystem (2) with the variable  $w = (H_2, X_{hy}, p_{H_2})$ . Let  $\lambda_{hy}$  denote  $\lambda_{hy}(a)$ , the unique solution of  $\mu_{hy}(H_2) = a$  (for the Monod function), where

$$a = m + \frac{D}{y_{X_{hy}}}.$$

Define

$$X_{hy}^* = \frac{D}{a} (H_2^{\text{in}} - \lambda_{hy}). \quad (15)$$

**Proposition 7.** Under hypotheses (H1–H3), Subsystem (2) admits two non-negative equilibria:

$$F_1 = (H_2^{\text{in}}, 0, h_{H_2} H_2^{\text{in}}), \quad F_2 = (\lambda_{hy}, X_{hy}^*, h_{H_2} \lambda_{hy}).$$

Moreover,  $F_1$  always exists, while  $F_2$  exists whenever  $X_{hy}^* \geq 0$  (i.e.,  $H_2^{\text{in}} \geq \lambda_{hy}$ ).

### 3.1.5. Jacobian Matrix of Subsystem (2)

Let  $w = (H_2, X_{hy}, p_{H_2})$  and set  $k = k_{L,H_2}$ ,  $h = h_{H_2}$  and  $\alpha = \frac{v_1}{v_g} rT$ . Denote  $\mu_{hy} = \mu_{hy}(H_2)$  and  $\mu'_{hy} = \mu'_{hy}(H_2)$ . The Jacobian matrix of Subsystem (2) at  $w$  is

$$J(w) = \begin{pmatrix} -D - k - \mu'_{hy} X_{hy} & -\mu_{hy} & \frac{k}{h} \\ y_{X_{hy}} \mu'_{hy} X_{hy} & y_{X_{hy}} (\mu_{hy} - m) - D & 0 \\ \alpha k & 0 & -\frac{\alpha k}{h} \end{pmatrix}. \quad (16)$$

**Lemma 2.** Let  $w = (H_2, X_{hy}, p_{H_2})$  and consider the Jacobian matrix  $J(w)$  defined in (16).

- **Case  $X_{hy} = 0$ .** If  $X_{hy} = 0$ , then by reordering the variables as  $(X_{hy}, H_2, p_{H_2})$ , the matrix becomes upper-triangular. Hence, its eigenvalues are

$$\lambda_6 = y_{X_{hy}} (\mu_{hy} - m) - D, \quad \lambda_{7,8} \text{ are the solutions of } \lambda^2 - \text{tr}(C)\lambda + \det(C) = 0, \quad (17)$$

where

$$C = \begin{pmatrix} -(D + k) & \frac{k}{h} \\ \alpha k & -\frac{\alpha k}{h} \end{pmatrix}, \quad \text{tr}(C) = -(D + k) - \frac{\alpha k}{h}, \quad \det(C) = \frac{\alpha k D}{h}. \quad (18)$$

- **Case  $X_{hy} \neq 0$ .** If  $X_{hy} \neq 0$ , then the eigenvalues of  $J(w)$  are the solutions of the characteristic equation

$$\lambda^3 + c_1 \lambda^2 + c_2 \lambda + c_3 = 0, \quad (19)$$

where  $c_1, c_2, c_3$  depend on  $w$  and on the model parameters.

**Proposition 8.** Assume that the equilibrium  $F_2 = (\lambda_{hy}, X_{hy}^*, h_{H_2} \lambda_{hy})$  exists. Then,  $F_2$  is locally asymptotically stable.

**Proof.** The proof is given in Appendix A.6.  $\square$

**Proposition 9.** The equilibrium  $F_1 = (H_2^{\text{in}}, 0, h_{H_2} H_2^{\text{in}})$  is locally asymptotically stable if and only if  $\mu_{hy}(H_2^{\text{in}}) < a$ .

**Proof.** The proof is given in Appendix A.7.  $\square$

Table 2 summarizes the existence and the local stability conditions of the equilibria of Subsystem (2).

**Table 2.** Existence and local stability of the equilibria of Subsystem (2).

| Equilibrium | Existence Condition                    | Local Stability                     |
|-------------|----------------------------------------|-------------------------------------|
| $F_1$       | always                                 | $H_2^{\text{in}} < \lambda_{hy}(a)$ |
| $F_2$       | $H_2^{\text{in}} \geq \lambda_{hy}(a)$ | S                                   |

### 3.1.6. Equilibria of the Full Model

Consider Subsystem (3) with the variable  $z = (CO_2, p_{CO_2}, CH_4, p_{CH_4})$ . For given values of  $(S_{dc}, X_a, S_{ac}, X_m, H_2, X_{hy})$ , we define

$$\begin{aligned}\Pi_{CO_2} &= (1 - y_{ac}) \frac{1 - y_a}{y_a} \mu_a(S_{dc}) X_a + y_{CO_2} \frac{1 - y_m}{y_m} \mu_m(S_{ac}) X_m - k_{CO_2} (\mu_{hy}(H_2) - m) X_{hy} - \frac{1}{4} m X_{hy}, \\ \Pi_{CH_4} &= (1 - y_{CO_2}) \frac{1 - y_m}{y_m} \mu_m(S_{ac}) X_m + k_{CH_4} (\mu_{hy}(H_2) - m) X_{hy} + \frac{1}{4} m X_{hy}.\end{aligned}\quad (20)$$

**Proposition 10.** Under hypotheses (H1–H4), for any equilibrium  $(S_{dc}^e, X_a^e, S_{ac}^e, X_m^e, H_2^e, X_{hy}^e, p_{H_2}^e)$  of Subsystem (1) and (2), Subsystem (3) admits a unique associated equilibrium  $z^e = (CO_2^e, p_{CO_2}^e, CH_4^e, p_{CH_4}^e)$ , given by

$$\begin{aligned}CO_2^e &= \frac{1}{D} (DCO_2^{\text{in}} + \Pi_{CO_2}|_e), & p_{CO_2}^e &= h_{CO_2} CO_2^e, \\ CH_4^e &= \frac{1}{D} (\Pi_{CH_4}|_e), & p_{CH_4}^e &= h_{CH_4} CH_4^e,\end{aligned}\quad (21)$$

where  $\Pi_{CO_2}|_e$  and  $\Pi_{CH_4}|_e$  denote Equation (20) at the corresponding equilibrium values. Then, the full model admits 12 non-negative equilibria of the form

$$\mathcal{E}_{ij} = (E_i, F_j, z_{ij}), \quad i = 1, \dots, 6, \quad j = 1, 2,$$

where  $z_{ij}$  is obtained from (21) by substituting  $(S_{dc}, X_a, S_{ac}, X_m, H_2, X_{hy})$  with the components of  $(E_i, F_j)$ .

### 3.1.7. Jacobian Structure of the Full Model

Let  $U = (u, w, z)$  denote the full-model variable, where

$$u = (S_s, S_{dc}, X_a, S_{ac}, X_m), \quad w = (H_2, X_{hy}, p_{H_2}), \quad z = (CO_2, p_{CO_2}, CH_4, p_{CH_4}).$$

As we remark that variables with  $z$  do not appear in the equations of  $(u, w)$ , the Jacobian matrix at any point  $U$  takes the form

$$J_{\text{full}}(U) = \begin{pmatrix} J_{(u,w)}(u, w) & 0 \\ \star & J_z(U) \end{pmatrix}, \quad (22)$$

where  $J_{(u,w)}$  is the Jacobian matrix of the previously studied subsystems, and  $J_z$  is the Jacobian matrix of Subsystem (3) with respect to  $z = (CO_2, p_{CO_2}, CH_4, p_{CH_4})$ .

Let  $k_1 = k_{L,CO_2}$ ,  $k_2 = h_{CO_2}$ ,  $k_3 = k_{L,CH_4}$ ,  $k_4 = h_{CH_4}$  and  $\alpha = \frac{v_1}{v_g} r T$ . We have

$$J_z(U) = \begin{pmatrix} -(D + k_1) & \frac{k_1}{k_2} & 0 & 0 \\ \alpha k_1 & -\frac{\alpha k_1}{k_2} & 0 & 0 \\ 0 & 0 & -(D + k_3) & \frac{k_3}{k_4} \\ 0 & 0 & \alpha k_3 & -\frac{\alpha k_3}{k_4} \end{pmatrix}.$$

Each  $2 \times 2$  block has a negative trace and positive determinant. Therefore, all eigenvalues of  $J_z$  have negative real parts.

Since the Jacobian matrix (22) is lower-triangular, its spectrum is the union of the spectra of  $J_{(u,w)}$  and  $J_z$ . Then, the local stability of each equilibrium

$$\mathcal{E}_{ij} = (E_i, F_j, z_{ij})$$

is determined by the stability of  $(E_i, F_j)$ .

### 3.2. Numerical Results

The objective of the numerical simulation is to use the analytical results obtained in the previous sections to characterize the operating behavior of the coupled system. More precisely, we present numerical simulations that illustrate the methane production at equilibrium as a function of the inflow concentrations and the dilution rate through operating diagrams. These diagrams are used to identify the operating conditions that are favorable for methane production. The numerical study also helps to determine, in bistable regions, which initial microbial concentrations lead the system toward the high methane equilibrium. The dilution rate and the inflow concentrations are considered as controllable operating variables, while the other model parameters are fixed.

The parameter values used in our numerical simulations are inspired by [14,24] and are presented in Table 3.

**Table 3.** Parameter values of the system.

| Subsystem     | Parameter      | Value                |
|---------------|----------------|----------------------|
| Subsystem (1) | $k_h$          | 0.2                  |
|               | $y_a$          | 0.15                 |
|               | $K_d$          | 1                    |
|               | $\mu_a^{\max}$ | 0.5                  |
|               | $y_{ac}$       | 0.8                  |
|               | $y_m$          | 0.06                 |
|               | $K_a$          | 1                    |
|               | $K_{ia}$       | 20                   |
|               | $\mu_m^{\max}$ | 0.6                  |
| Subsystem (2) | $q^{\max}$     | 1                    |
|               | $K_{H_2}$      | $5.6 \times 10^{-3}$ |
|               | $k_{L,H_2}$    | 50                   |
|               | $h_{H_2}$      | $1.3 \times 10^5$    |
|               | $y_{x_{hy}}$   | 0.5                  |
|               | $m$            | $5 \times 10^{-3}$   |
|               | $v_l$          | 1                    |
|               | $v_g$          | 0.2                  |
|               | $r$            | 8.314                |
|               | $T$            | 308                  |
| Subsystem (3) | $y_{CO_2}$     | 0.3                  |
|               | $k_{CO_2}$     | 0.8                  |
|               | $k_{L,CO_2}$   | 33                   |
|               | $h_{CO_2}$     | $3 \times 10^5$      |
|               | $k_{CH_4}$     | 1.2                  |
|               | $k_{L,CH_4}$   | 29                   |
|               | $h_{CH_4}$     | $7 \times 10^4$      |

#### 3.2.1. Numerical Procedures

All numerical simulations in this study were performed using MATLAB R2018a (version 9.4.0.813654). The operating diagrams and methane production maps were constructed

from the analytical existence and local stability conditions derived in the previous sections. The method used to generate these figures can be summarized as follows:

1. Generate a two-dimensional grid for the selected operating parameters, such as  $S_{ac}^{\text{in}}$  and  $D$ .
2. For each value of  $D$ , compute the threshold functions  $\lambda_a(D)$ ,  $\lambda_m^-(D)$ ,  $\lambda_m^+(D)$ , and  $\lambda_{hy}(D)$ .
3. Determine the AD region  $A_i$  and the hydrogenotrophic biomethanation region  $B_j$ .
4. Combine  $A_i$  and  $B_j$  to assign the corresponding coupled region  $R_k$ .
5. Identify the existing and locally stable coupled equilibria  $(E_i, F_j)$ .
6. Compute the equilibrium methane concentration  $CH_4^e$  for each locally stable equilibrium.
7. If there are multiple stable equilibria, find the maximum and minimum values of  $CH_4^e$ ; otherwise, find the single value.
8. Plot the coupled operating regions, the number of stable equilibria, and the methane production maps.

For the basin of attraction simulations, the coupled system was solved using the MATLAB solver ode15s. Relative and absolute tolerances were set to  $10^{-7}$  and  $10^{-10}$ , respectively. The system was simulated over the time interval  $[0, 2000]$ . The initial values of  $S_{ac}(0)$  and  $X_m(0)$  were varied over a two-dimensional grid. For each initial condition, the final state of the solution was compared to the locally stable equilibria, and the initial condition was assigned to the basin of the equilibrium toward which the trajectory converged.

### 3.2.2. Operating Diagrams and Methane Production Maps

Before presenting the operating regions, we recall that the threshold substrate concentrations associated with the growth functions depend on the dilution rate  $D$ , while the equilibrium substrate concentrations depend on both the influent substrate concentrations and  $D$ :

$$\lambda_a(D) = \frac{K_a D}{\mu_a^{\text{max}} - D}, \quad \lambda_m^{\pm}(D) = \frac{K_{ia}}{2D} \left( \mu_m^{\text{max}} - D \pm \sqrt{(\mu_m^{\text{max}} - D)^2 - \frac{4K_a D^2}{K_{ia}}} \right)$$

and

$$S_{dc}^*(D) = S_{dc}^{\text{in}} + \frac{k_h}{k_h + D} S_s^{\text{in}}, \quad S_{ac}^*(D) = S_{ac}^{\text{in}} + y_{ac}(1 - y_a)(S_{dc}^*(D) - \lambda_a(D)).$$

Using the existence and the local stability conditions of Table 1, together with the monotonicity of the Monod function  $\mu_a$  and the non-monotonicity of the Haldane function  $\mu_m$ , the operating regions of Subsystem (1) can be classified as in Tables 4 and 5.

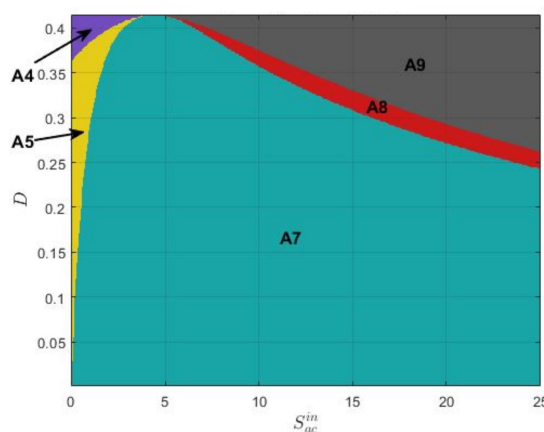
**Table 4.** Regions of the operating diagram of Subsystem (1) in the case  $S_{dc}^* < \lambda_a$ . S: Stable; U: unstable; no sign: non-existence.

| Region | Conditions                                                                   | $E_1$ | $E_2$ | $E_3$ | $E_4$ | $E_5$ | $E_6$ |
|--------|------------------------------------------------------------------------------|-------|-------|-------|-------|-------|-------|
| $A_1$  | $S_{dc}^* < \lambda_a, \quad S_{ac}^{\text{in}} < \lambda_m^-$               | S     |       |       |       |       |       |
| $A_2$  | $S_{dc}^* < \lambda_a, \quad \lambda_m^- < S_{ac}^{\text{in}} < \lambda_m^+$ | U     |       | S     |       |       |       |
| $A_3$  | $S_{dc}^* < \lambda_a, \quad \lambda_m^+ < S_{ac}^{\text{in}}$               | S     |       | S     | U     |       |       |

**Table 5.** Regions of the operating diagram of Subsystem (1) in the case  $S_{dc}^* > \lambda_a$ . S: Stable; U: unstable; no sign: non-existence.

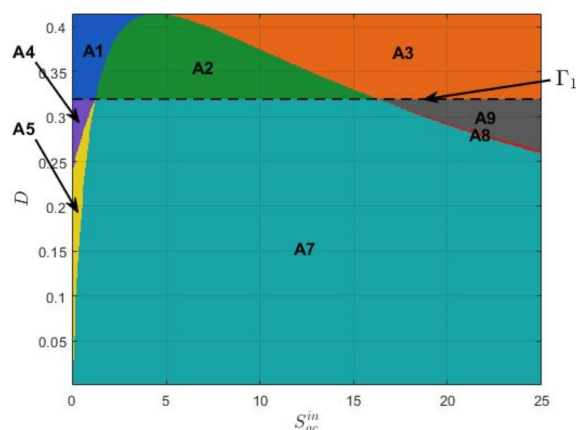
| Region | Conditions                                                                 | $E_1$ | $E_2$ | $E_3$ | $E_4$ | $E_5$ | $E_6$ |
|--------|----------------------------------------------------------------------------|-------|-------|-------|-------|-------|-------|
| $A_4$  | $S_{dc}^* > \lambda_a, S_{ac}^{in} < S_{ac}^* < \lambda_m^-$               | U     | S     |       |       |       |       |
| $A_5$  | $S_{dc}^* > \lambda_a, S_{ac}^{in} < \lambda_m^- < S_{ac}^* < \lambda_m^+$ | U     | U     |       |       | S     |       |
| $A_6$  | $S_{dc}^* > \lambda_a, S_{ac}^{in} < \lambda_m^- < \lambda_m^+ < S_{ac}^*$ | U     | S     |       |       | S     | U     |
| $A_7$  | $S_{dc}^* > \lambda_a, \lambda_m^- < S_{ac}^{in} < S_{ac}^* < \lambda_m^+$ | U     | U     | U     |       | S     |       |
| $A_8$  | $S_{dc}^* > \lambda_a, \lambda_m^- < S_{ac}^{in} < \lambda_m^+ < S_{ac}^*$ | U     | S     | U     |       | S     | U     |
| $A_9$  | $S_{dc}^* > \lambda_a, \lambda_m^+ < S_{ac}^{in} < S_{ac}^*$               | U     | S     | U     | U     | S     | U     |

Figure 2 illustrates the operating diagram of Subsystem (1) in the  $(S_{ac}^{in}, D)$  plane, while the other inflow concentrations are fixed. For a high fixed value of the inflow concentration  $S_s^{in} = 12$ , the condition  $S_{dc}^* > \lambda_a$  is satisfied. This means that the dissolved organic substrate can be consumed by the acidogenic bacteria. Therefore, according to Table 4, regions  $A_1$ ,  $A_2$ , and  $A_3$  do not appear in this diagram. The operating space is then divided into five regions,  $A_4$ ,  $A_5$ ,  $A_7$ ,  $A_8$ , and  $A_9$ . We observe that region  $A_7$  occupies the largest part of the diagram. This region is bounded by the curve  $S_{ac}^{in} = \lambda_m^-(D)$ , which separates it from  $A_5$ , and by the curve  $S_{ac}^*(D) = \lambda_m^+(D)$ , which separates it from  $A_8$ . In this region, the acetate concentrations remain between the two Haldane thresholds  $\lambda_m^-$  and  $\lambda_m^+$ . Hence, according to Table 5, the stable equilibrium is  $E_5$ . This equilibrium corresponds to a favorable situation for methane production, since both acidogenic and methanogenic bacteria are maintained. However, when the dilution rate  $D$  increases, the interval between  $\lambda_m^-$  and  $\lambda_m^+$  becomes smaller. This explains why the region  $A_7$  reduces when the values of  $D$  are high. For small values of  $S_{ac}^{in}$ , region  $A_5$  appears on the left side of  $A_7$ . It is bounded by the curve  $S_{ac}^{in} = \lambda_m^-(D)$  and by the curve  $S_{ac}^*(D) = \lambda_m^-(D)$ , which separates it from  $A_4$ . In  $A_5$ , although the inflow acetate concentration satisfies  $S_{ac}^{in} < \lambda_m^-$ , the value of  $S_{ac}^*$  remains between  $\lambda_m^-$  and  $\lambda_m^+$ . Thus, as in  $A_7$ , the stable equilibrium is  $E_5$ . In contrast, for larger dilution rates and small values of  $S_{ac}^{in}$ , region  $A_4$  is obtained. In this case,  $S_{ac}^*$  becomes smaller than  $\lambda_m^-$ , which leads to the washout of the methanogenic bacteria. The stable equilibrium is then  $E_2$ , and the methane production through this subsystem is not maintained. On the right side of  $A_7$ , regions  $A_8$  and  $A_9$  appear. These two regions are associated with the inhibition described by the Haldane function. They are located above the curve  $S_{ac}^*(D) = \lambda_m^+(D)$ , where high acetate levels may inhibit methanogenic growth. According to Table 5, these regions correspond to bistability between  $E_2$  and  $E_5$ . The curve  $S_{ac}^{in} = \lambda_m^+(D)$  separates  $A_8$  from  $A_9$ . In region  $A_9$ , the equilibrium  $E_4$  also exists, but it is unstable.



**Figure 2.** Operating diagram in the plane  $(S_{ac}^{in}, D)$  for  $S_s^{in} = 12$  and  $S_{dc}^{in} = 1$ .

Figure 3 illustrates the operating diagram of Subsystem (1) also in the  $(S_{ac}^{in}, D)$  plane, but for a smaller fixed value of the inflow concentration  $S_s^{in} = 2$ . In this case, the condition  $S_{dc}^* > \lambda_a$  is not always satisfied. This leads to the appearance of the horizontal boundary  $\Gamma_1$ , which is characterized by  $S_{dc}^*(D) = \lambda_a(D)$ . Below this boundary, we have  $S_{dc}^* > \lambda_a$ , and the same regions observed in Figure 2 appear. However, these regions become smaller compared to Figure 2. Above  $\Gamma_1$ , we have  $S_{dc}^* < \lambda_a$ , and the new regions  $A_1$ ,  $A_2$ , and  $A_3$  appear. These new regions appear because the smaller value of  $S_s^{in}$  may make  $S_{dc}^*$  insufficient to maintain the acidogenic bacteria. Since  $\lambda_a(D)$  increases with  $D$ , the condition  $S_{dc}^* < \lambda_a$  is obtained for large dilution rates. This explains the appearance of  $A_1$ ,  $A_2$ , and  $A_3$  above  $\Gamma_1$ . In region  $A_1$ , the inflow acetate concentration satisfies  $S_{ac}^{in} < \lambda_m^-$ . This region is located on the left side of the curve  $S_{ac}^{in} = \lambda_m^-(D)$ . In this case, both acidogenic and methanogenic bacteria are not maintained, and the stable equilibrium is the washout equilibrium  $E_1$ . Region  $A_2$  is located between the two curves  $S_{ac}^{in} = \lambda_m^-(D)$  and  $S_{ac}^{in} = \lambda_m^+(D)$ . In this region, the acidogenic bacteria are washed out because  $S_{dc}^* < \lambda_a$ , but the methanogenic bacteria can still be maintained due to the acetate inflow. Therefore, the stable equilibrium is  $E_3$ . On the right side of the curve  $S_{ac}^{in} = \lambda_m^+(D)$ , region  $A_3$  appears. In this region, the acetate inflow is high, and the acidogenic bacteria are still washed out. Because of the Haldane inhibition effect, the system may converge either to the washout equilibrium  $E_1$  or to the equilibrium  $E_3$ , depending on the initial conditions. Thus,  $A_3$  corresponds to a bistable region between  $E_1$  and  $E_3$ .



**Figure 3.** Operating diagram in the plane  $(S_{ac}^{in}, D)$  for  $S_s^{in} = 2$  and  $S_{dc}^{in} = 1$ .

Using the existence and the local stability conditions of Table 2, together with the monotonicity of the Monod function  $\mu_{hy}$ , the operating regions of Subsystem (2) can be classified as in Table 6.

**Table 6.** Regions of the operating diagram for Subsystem (2). S: Stable; U: unstable; no sign: non-existence.

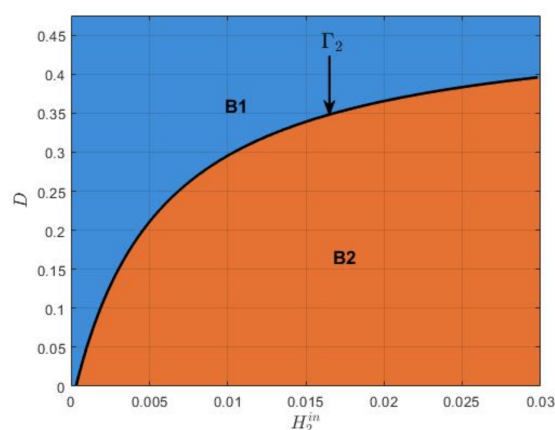
| Region | Conditions                | $F_1$ | $F_2$ |
|--------|---------------------------|-------|-------|
| $B_1$  | $H_2^{in} < \lambda_{hy}$ | S     |       |
| $B_2$  | $H_2^{in} > \lambda_{hy}$ | U     | S     |

Figure 4 presents the operating diagram of Subsystem (2) in the  $(H_2^{in}, D)$  plane. Due to the Monod growth function of the hydrogenotrophic bacteria, the operating space is

divided into two regions separated by the black boundary curve  $\Gamma_2$ , which is characterized by  $H_2^{\text{in}} = \lambda_{hy}(D)$ , with

$$\lambda_{hy}(D) = \frac{K_{H_2} \left( m + \frac{D}{y_{X_{hy}}} \right)}{q^{\text{max}} - \left( m + \frac{D}{y_{X_{hy}}} \right)}.$$

Above this boundary, we have  $H_2^{\text{in}} < \lambda_{hy}$ . Thus, the hydrogen concentration is not sufficient to maintain the hydrogenotrophic bacteria, and region  $B_1$  is obtained. According to Table 6, the unique stable equilibrium in this region is  $F_1$ , which corresponds to the washout of the hydrogenotrophic bacteria. In this case, the hydrogen is not biologically consumed, and no methane is produced through Subsystem (2). Below the boundary curve, we have  $H_2^{\text{in}} > \lambda_{hy}$ , and region  $B_2$  appears. In this region, equilibrium  $F_1$  becomes unstable, while equilibrium  $F_2$  is stable. This operating region is characterized by the endurance of the hydrogenotrophic bacteria, leading to hydrogen consumption and methane production through the hydrogenotrophic pathway.



**Figure 4.** Operating diagram in the plane  $(H_2^{\text{in}}, D)$ .

By combining the operating regions of Subsystem (1) (Tables 4 and 5) with those in Table 6 of Subsystem (2), the operating regions of the coupled system can be classified as indicated in Tables 7 and 8. Since Subsystem (1) admits nine operating regions and Subsystem (2) admits two regions, the coupled system naturally gives  $9 \times 2 = 18$  possible regions.

**Table 7.** Regions of the operating diagram of the coupled system in the case  $H_2^{\text{in}} < \lambda_{hy}$  (in region  $B_1$ ). S: Stable; U: unstable; no sign: non-existence.

| Region | Conditions                            | $(E_1, F_1)$ | $(E_1, F_2)$ | $(E_2, F_1)$ | $(E_2, F_2)$ | $(E_3, F_1)$ | $(E_3, F_2)$ | $(E_4, F_1)$ | $(E_4, F_2)$ | $(E_5, F_1)$ | $(E_5, F_2)$ | $(E_6, F_1)$ | $(E_6, F_2)$ |
|--------|---------------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| $R_1$  | $A_1, H_2^{\text{in}} < \lambda_{hy}$ | S            |              |              |              |              |              |              |              |              |              |              |              |
| $R_2$  | $A_2, H_2^{\text{in}} < \lambda_{hy}$ | U            |              |              |              | S            |              |              |              |              |              |              |              |
| $R_3$  | $A_3, H_2^{\text{in}} < \lambda_{hy}$ | S            |              |              |              | S            |              | U            |              |              |              |              |              |
| $R_4$  | $A_4, H_2^{\text{in}} < \lambda_{hy}$ | U            |              | S            |              |              |              |              |              |              |              |              |              |
| $R_5$  | $A_5, H_2^{\text{in}} < \lambda_{hy}$ | U            |              | U            |              |              |              |              |              | S            |              |              |              |
| $R_6$  | $A_6, H_2^{\text{in}} < \lambda_{hy}$ | U            |              | S            |              |              |              |              |              | S            |              | U            |              |
| $R_7$  | $A_7, H_2^{\text{in}} < \lambda_{hy}$ | U            |              | U            |              | U            |              |              |              | S            |              |              |              |
| $R_8$  | $A_8, H_2^{\text{in}} < \lambda_{hy}$ | U            |              | S            |              | U            |              |              |              | S            |              | U            |              |
| $R_9$  | $A_9, H_2^{\text{in}} < \lambda_{hy}$ | U            |              | S            |              | U            |              | U            |              | S            |              | U            |              |

**Table 8.** Regions of the operating diagram of the coupled system in the case  $H_2^{\text{in}} > \lambda_{hy}$  (in region  $B_2$ ). S: Stable; U: unstable; no sign: non-existence.

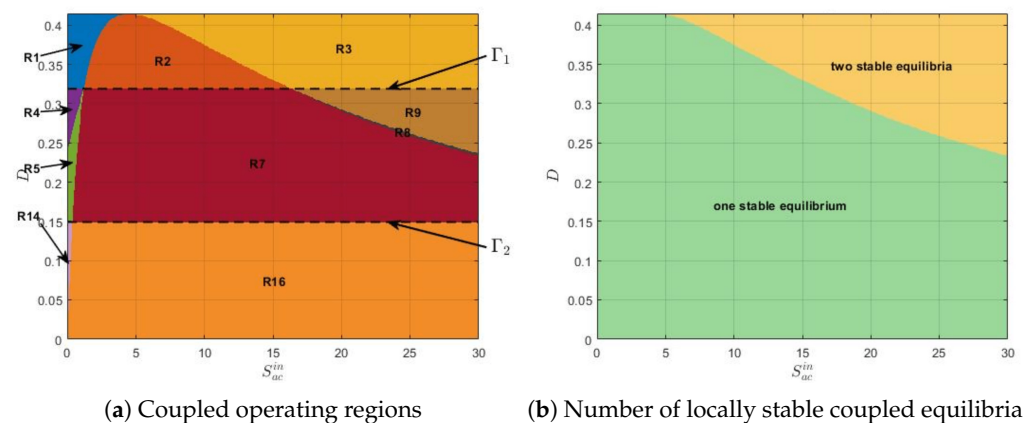
| Region   | Conditions                            | $(E_1, F_1)$ | $(E_1, F_2)$ | $(E_2, F_1)$ | $(E_2, F_2)$ | $(E_3, F_1)$ | $(E_3, F_2)$ | $(E_4, F_1)$ | $(E_4, F_2)$ | $(E_5, F_1)$ | $(E_5, F_2)$ | $(E_6, F_1)$ | $(E_6, F_2)$ |
|----------|---------------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| $R_{10}$ | $A_1, H_2^{\text{in}} > \lambda_{hy}$ | U            | S            |              |              |              |              |              |              |              |              |              |              |
| $R_{11}$ | $A_2, H_2^{\text{in}} > \lambda_{hy}$ | U            | U            |              |              | U            | S            |              |              |              |              |              |              |
| $R_{12}$ | $A_3, H_2^{\text{in}} > \lambda_{hy}$ | U            | S            |              |              | U            | S            | U            | U            |              |              |              |              |
| $R_{13}$ | $A_4, H_2^{\text{in}} > \lambda_{hy}$ | U            | U            | U            | S            |              |              |              |              |              |              |              |              |
| $R_{14}$ | $A_5, H_2^{\text{in}} > \lambda_{hy}$ | U            | U            | U            | U            |              |              |              |              | U            | S            |              |              |
| $R_{15}$ | $A_6, H_2^{\text{in}} > \lambda_{hy}$ | U            | U            | U            | S            |              |              |              |              | U            | S            | U            | U            |
| $R_{16}$ | $A_7, H_2^{\text{in}} > \lambda_{hy}$ | U            | U            | U            | U            | U            | U            |              |              | U            | S            |              |              |
| $R_{17}$ | $A_8, H_2^{\text{in}} > \lambda_{hy}$ | U            | U            | U            | S            | U            | U            |              |              | U            | S            | U            | U            |
| $R_{18}$ | $A_9, H_2^{\text{in}} > \lambda_{hy}$ | U            | U            | U            | S            | U            | U            | U            | U            | U            | S            | U            | U            |

In the following, we choose the  $(S_{ac}^{\text{in}}, D)$  plane for the operating diagram of the coupled system, as the acetate input plays a direct role in the methanogenic step. This plane also allows us to identify the regions associated with the Haldane thresholds  $\lambda_m^-$  and  $\lambda_m^+$ , which lead to the appearance of favorable operating regions and bistable regions. Consequently, this representation is more appropriate for discussing methane production.

Figure 5a presents the operating diagram of the coupled system formed by Subsystems (1) and (2) in the  $(S_{ac}^{\text{in}}, D)$  plane for relatively small fixed values of the inflow concentrations  $S_s^{\text{in}} = 2$  and  $H_2^{\text{in}} = 0.003$ . Figure 5b shows the corresponding number of stable coupled equilibria in each operating region. The value of  $S_s^{\text{in}}$  is the same as in Figure 3, while the fixed hydrogen inflow concentration introduces the additional horizontal boundary  $\Gamma_2$ , defined by  $H_2^{\text{in}} = \lambda_{hy}(D)$ . Above  $\Gamma_2$ , we have  $H_2^{\text{in}} < \lambda_{hy}$ . Then, the hydrogen concentration is not sufficient to maintain the hydrogenotrophic bacteria, and the stable equilibria are associated with  $F_1$ . In this part of the diagram, the same type of region as in Figure 3 appears, but each equilibrium of Subsystem (1) is coupled with  $F_1$ . Thus, the large region between  $\Gamma_1$  and  $\Gamma_2$  is  $R_7$ , where the stable equilibrium is  $(E_5, F_1)$ . This region corresponds to the methane production through the AD, since the acidogenic and the methanogenic bacteria are maintained. However, the hydrogenotrophic bacteria are washed out, so there is no additional methane production through the hydrogenotrophic pathway. The bistable regions  $R_8$  and  $R_9$  also appear above  $\Gamma_2$ . They are characterized by the coexistence of two stable equilibria,  $(E_2, F_1)$  and  $(E_5, F_1)$ . In these regions, the system may converge either to a regime without methane production by methanogenic bacteria or to a regime where the methane production is maintained through the AD. For large dilution rates, the condition  $S_{dc}^* < \lambda_a$  also holds. This corresponds to the part above  $\Gamma_1$ , where regions  $R_1$ ,  $R_2$ , and  $R_3$  appear. In  $R_1$ , the stable equilibrium is  $(E_1, F_1)$ , which corresponds to the washout of all microbial populations. In  $R_2$ , the stable equilibrium is  $(E_3, F_1)$ , where the acidogenic and hydrogenotrophic populations are washed out, while the methanogenic bacteria may be maintained by the acetate inflow. In  $R_3$ , the system is bistable between  $(E_1, F_1)$  and  $(E_3, F_1)$ , and the long-term behavior depends on the initial conditions. Below  $\Gamma_2$ , we have  $H_2^{\text{in}} > \lambda_{hy}$ . This condition is obtained for small dilution rates because the Monod threshold  $\lambda_{hy}(D)$  is smaller when  $D$  is small. In this case, the hydrogenotrophic bacteria can be maintained. Since this part is also below  $\Gamma_1$ , the condition  $S_{dc}^* > \lambda_a$  holds, and the regions related to the washout of acidogenic bacteria do not appear. Therefore, only regions  $R_{14}$  and  $R_{16}$  are observed in this range of the diagram. According to Table 8, both  $R_{14}$  and  $R_{16}$  are characterized by the stable coupled equilibrium  $(E_5, F_2)$ . This equilibrium corresponds biologically to the coexistence of the three microbial populations. Therefore,

these regions represent a favorable operating situation, with substrate degradation, hydrogen consumption, and methane production through both AD and biomethanation. The difference between  $R_{14}$  and  $R_{16}$  is related to the boundary  $S_{ac}^{in} = \lambda_m^-(D)$ . In  $R_{16}$ , some additional equilibria exist, but they are unstable and do not change the long-term stable behavior.

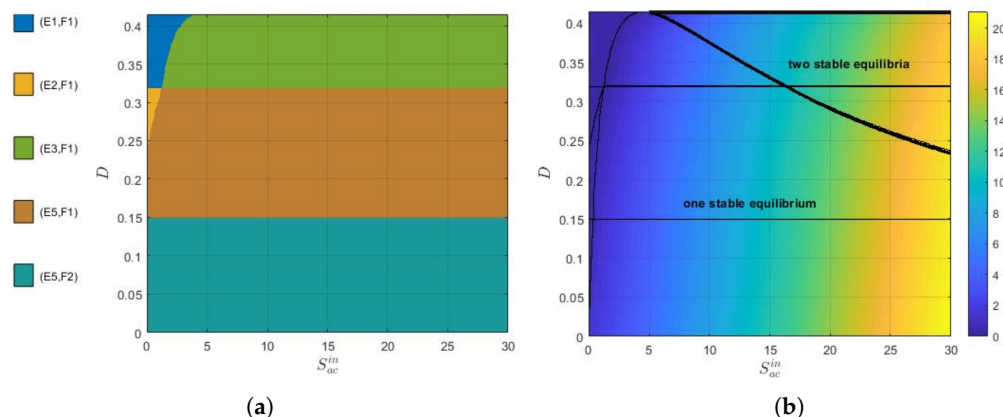
Figure 6a identifies, in each operating region, the stable coupled equilibrium that provides the highest methane concentration. This analysis is particularly important in the bistable regions  $R_3$ ,  $R_8$ , and  $R_9$ , where two stable equilibria coexist. In region  $R_3$ , the system admits two stable equilibria,  $(E_1, F_1)$  and  $(E_3, F_1)$ . Since  $(E_1, F_1)$  corresponds to total washout, the greatest methane production is obtained at  $(E_3, F_1)$ , where the methanogenic bacteria can still be maintained by the acetate inflow. However, this production remains limited because the acidogenic and hydrogenotrophic populations are washed out. Similarly, in regions  $R_8$  and  $R_9$ , bistability occurs between  $(E_2, F_1)$  and  $(E_5, F_1)$ . The equilibrium  $(E_5, F_1)$  gives the highest methane level because both the acidogenic and methanogenic populations are maintained. In contrast,  $(E_2, F_1)$  corresponds to the washout of methanogenic bacteria and therefore gives very low methane production. In the remaining regions, only one stable equilibrium exists. In particular, regions  $R_{14}$  and  $R_{16}$  are characterized by the stable equilibrium  $(E_5, F_2)$ , where the three microbial populations coexist. This is the most favorable biological situation since methane can be produced through both AD and the hydrogenotrophic pathway. As shown in Figure 6b, methane production increases with the acetate inflow concentration  $S_{ac}^{in}$  and can reach high values in region  $R_{16}$ . In region  $R_7$ , the unique stable equilibrium is  $(E_5, F_1)$ . Although the hydrogenotrophic population is washed out in this region, methane production remains significant due to the activity of the acidogenic and methanogenic populations. However, since the hydrogenotrophic pathway does not contribute to the methane production, a larger value of  $S_{ac}^{in}$  is required to reach methane levels comparable to those obtained in regions where  $F_2$  is stable.



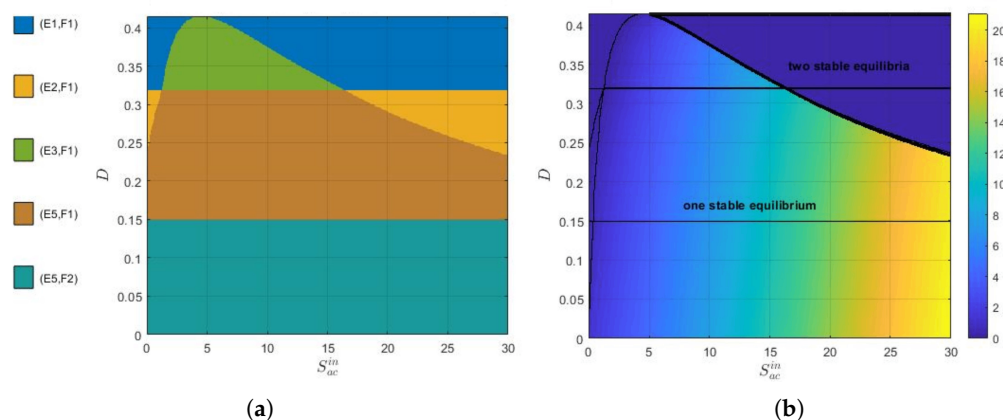
**Figure 5.** Coupled operating regions in the  $(S_{ac}^{in}, D)$  plane for  $S_s^{in} = 2$ ,  $S_{dc}^{in} = 1$  and  $H_2^{in} = 0.003$ .

Figure 7 complements the previous analysis by identifying, in each operating region, the stable coupled equilibrium that yields the minimum methane concentration as observed in Figure 7a. This information is particularly significant in bistable regions such as  $R_3$ ,  $R_8$ , and  $R_9$ . In region  $R_3$ , the lowest methane production is associated with the equilibrium  $(E_1, F_1)$ , which corresponds to total washout and therefore to the absence of biological methane generation. Similarly, in regions  $R_8$  and  $R_9$ , the equilibrium  $(E_2, F_1)$  provides the minimum methane level, since it corresponds to the loss of the methanogenic biomass. As illustrated in Figure 7b, the methane levels in regions with a single stable equilibrium follow the same direction as in Figure 6b. In contrast, in the bistable regions, the minimum methane

production is always associated with equilibria giving the methanogenic loss, specifically  $(E_1, F_1)$  and  $(E_2, F_1)$ . In these regions, the global methane yield becomes nearly zero.



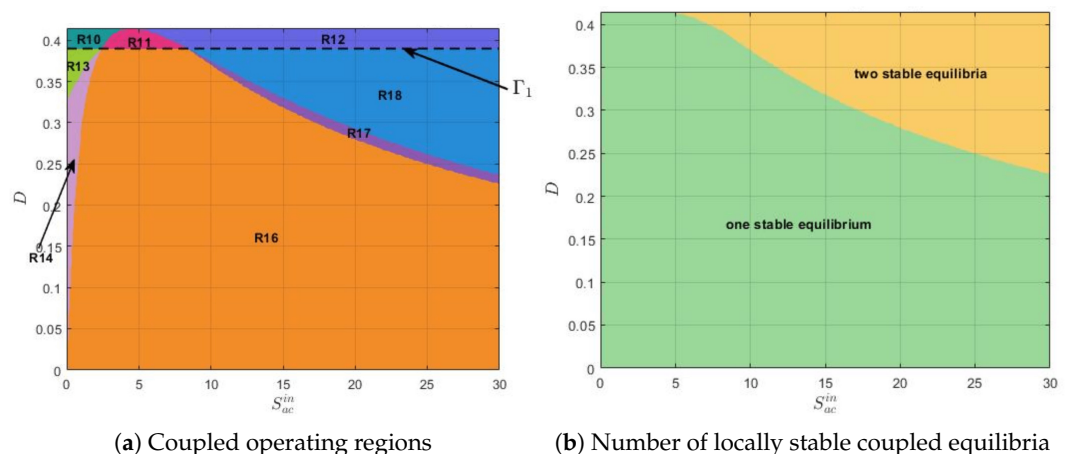
**Figure 6.** Methane production performance in the  $(S_{ac}^{in}, D)$  plane for  $S_s^{in} = 2$ ,  $S_{dc}^{in} = 1$  and  $H_2^{in} = 0.003$ . (a) Stable equilibrium yielding the highest methane level. (b) Maximum methane concentration  $CH_4^e$ .



**Figure 7.** Worst methane production performance in the  $(S_{ac}^{in}, D)$  plane for  $S_s^{in} = 2$ ,  $S_{dc}^{in} = 1$  and  $H_2^{in} = 0.003$ . (a) Stable equilibrium yielding the lowest methane level. (b) Minimum methane concentration  $CH_4^e$ .

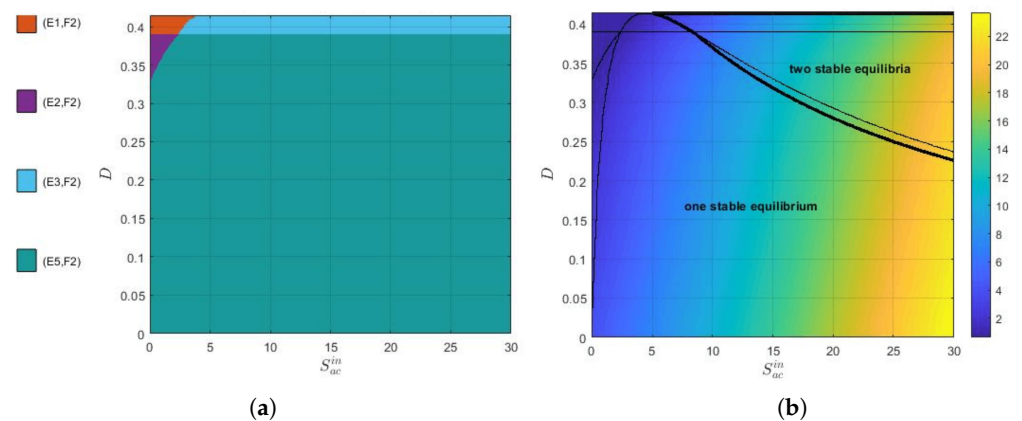
Figure 8a presents the operating diagram of the coupled system in the  $(S_{ac}^{in}, D)$  plane for fixed values of the inflow concentrations  $S_s^{in} = 7.5$  and  $H_2^{in} = 0.6$ . Figure 8b shows the corresponding number of stable coupled equilibria in each operating region. Compared with Figure 5a, the value of  $S_s^{in}$  is larger. Therefore, the condition  $S_{dc}^* > \lambda_a$  is satisfied for a larger part of the diagram, and the boundary  $\Gamma_1$ , defined by  $S_{dc}^*(D) = \lambda_a(D)$ , appears only for large values of the dilution rate  $D$ . This is due to the Monod threshold  $\lambda_a(D)$ , which increases with  $D$ . In this configuration, the hydrogen inflow concentration is high enough to satisfy  $H_2^{in} > \lambda_{hy}$  in the considered range of  $D$ . Thus, the boundary  $\Gamma_2$  does not appear in the diagram. The hydrogenotrophic bacteria are maintained, and the stable equilibria are associated with  $F_2$ . Below  $\Gamma_1$ , we have  $S_{dc}^* > \lambda_a$ . The large region in this part of the diagram is  $R_{16}$ , where the stable equilibrium is  $(E_5, F_2)$ . This equilibrium corresponds to a favorable situation, since the acidogenic, methanogenic, and hydrogenotrophic populations are all maintained. Methane can therefore be produced through both AD and the hydrogenotrophic pathway. The small regions  $R_{13}$  and  $R_{14}$  also appear for small values of  $S_{ac}^{in}$ . They correspond to cases where the acetate level is close to the lower Haldane threshold. In these regions, the hydrogenotrophic biomass is still maintained because the equilibrium is coupled with  $F_2$ . For larger values of  $S_{ac}^{in}$ , the bistable regions  $R_{17}$  and  $R_{18}$  appear below  $\Gamma_1$ . They are characterized by the coexistence of two stable equilibria,  $(E_2, F_2)$  and  $(E_5, F_2)$ . In

the equilibrium  $(E_2, F_2)$ , the methanogenic bacteria of the AD step are washed out, while the hydrogenotrophic population is maintained. In contrast,  $(E_5, F_2)$  corresponds to the maintenance of the three microbial populations. Thus, these regions may lead either to a reduced methane-producing state or to a more favorable state with methane production through the full coupled system. Therefore, the final state depends on the basin of attraction of the initial condition. Above  $\Gamma_1$ , we have  $S_{dc}^* < \lambda_a$ , which means that the acidogenic bacteria cannot be maintained. In this part of the diagram, regions  $R_{10}$ ,  $R_{11}$ , and  $R_{12}$  appear. In  $R_{10}$ , the stable equilibrium is  $(E_1, F_2)$ , corresponding to the washout of the acidogenic and methanogenic populations, while the hydrogenotrophic biomass remains maintained. In  $R_{11}$ , the stable equilibrium is  $(E_3, F_2)$ , where the acidogenic population is washed out, but the methanogenic bacteria can be maintained by the acetate inflow, together with the hydrogenotrophic bacteria. Finally,  $R_{12}$  is a bistable region between  $(E_1, F_2)$  and  $(E_3, F_2)$ .



**Figure 8.** Coupled operating regions in the  $(S_{ac}^{in}, D)$  plane for  $S_s^{in} = 7.5$ ,  $S_{dc}^{in} = 1$  and  $H_2^{in} = 0.6$ .

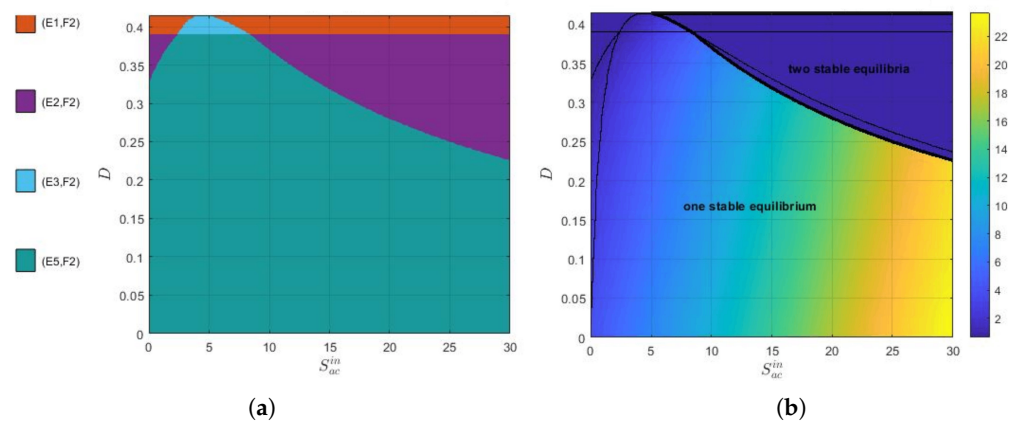
Figure 9a identifies, in each operating region, the stable coupled equilibrium that provides the highest methane concentration. This analysis is particularly important in the bistable regions  $R_{12}$ ,  $R_{17}$ , and  $R_{18}$ , where two stable equilibria coexist. In region  $R_{12}$ , the system admits two stable equilibria,  $(E_1, F_2)$  and  $(E_3, F_2)$ . Since  $(E_1, F_2)$  corresponds to the washout of the AD bacteria, the greatest methane production is obtained at  $(E_3, F_2)$ , where methanogenic bacteria can still be maintained by the acetate inflow and the hydrogenotrophic bacteria are maintained by the hydrogen inflow. However, this situation is not the most favorable one because the acidogenic population is washed out. Similarly, in regions  $R_{17}$  and  $R_{18}$ , bistability occurs between  $(E_2, F_2)$  and  $(E_5, F_2)$ . The equilibrium  $(E_5, F_2)$  gives the highest methane level because all microbial populations are maintained. In contrast,  $(E_2, F_2)$  corresponds to the washout of the methanogenic bacteria of the AD step. Therefore,  $(E_5, F_2)$  represents the most favorable biological situation, since methane can be produced through both anaerobic digestion and the hydrogenotrophic pathway. As shown in Figure 9b, methane production increases with the acetate inflow concentration  $S_{ac}^{in}$ . High methane production is obtained in region  $R_{16}$ , where  $(E_5, F_2)$  is the unique stable equilibrium. In the bistable regions  $R_{17}$  and  $R_{18}$ , high methane production is also possible through the equilibrium  $(E_5, F_2)$ , but another stable equilibrium with lower methane production also exists. For larger dilution rates, a higher value of  $S_{ac}^{in}$  is generally needed to compensate the dilution effect and maintain a high methane level.



**Figure 9.** Methane production performance in the  $(S_{ac}^{in}, D)$  plane for  $S_s^{in} = 7.5$ ,  $S_{dc}^{in} = 1$  and  $H_2^{in} = 0.6$ . (a) Stable equilibrium yielding the highest methane level. (b) Maximum methane concentration  $CH_4^e$ .

Figure 10 completes the methane production analysis for large values of  $S_s^{in}$  and  $H_2^{in}$ . Figure 10a identifies, in each operating region, the stable coupled equilibrium that yields the minimum methane concentration. This analysis is mainly important in the bistable regions  $R_{12}$ ,  $R_{17}$ , and  $R_{18}$ . In region  $R_{12}$ , the equilibrium  $(E_1, F_2)$  is associated with the lowest methane production. This equilibrium corresponds to the washout of the acidogenic and methanogenic populations, while the hydrogenotrophic bacteria are still maintained. Therefore, methane production is reduced and comes only from the hydrogenotrophic pathway. Similarly, in regions  $R_{17}$  and  $R_{18}$ , bistability occurs between  $(E_2, F_2)$  and  $(E_5, F_2)$ . The minimum methane level is obtained at  $(E_2, F_2)$ , since this equilibrium reflects the loss of the methanogenic bacteria in the AD step. In this case, the methane production is lower than that obtained at  $(E_5, F_2)$ , where all microbial populations are maintained. As illustrated in Figure 10b, in the regions with a single stable equilibrium, the methane level follows the same direction as in Figure 9b. In particular, the large lower part of the diagram remains associated with  $(E_5, F_2)$  even for the minimum methane-producing equilibrium. This means that this is the favorable operating zone, since methane production is maintained through both AD and the hydrogenotrophic pathway. In contrast, in the bistable regions, the minimum methane-producing state is associated with equilibria including the loss of the anaerobic methanogenic population, specifically  $(E_1, F_2)$  or  $(E_2, F_2)$ . Thus, even when the hydrogenotrophic pathway remains active, the global methane yield may be strongly reduced if the system reaches one of these low-production equilibria.

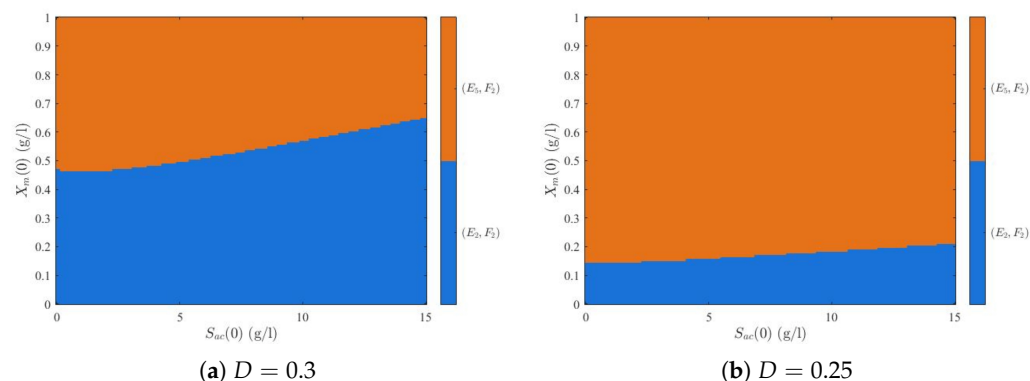
These results provide a practical interpretation of the 12 equilibria and the 18 operating regions of the system. To obtain a favorable methane production, the inflow concentrations should be selected in such a way to maintain the microbial populations. First, the biodegradable solid or dissolved substrate inflow concentration should be enough to satisfy  $S_{dc}^* > \lambda_a$  to avoid the washout of acidogenic bacteria. Second, the hydrogen inflow concentration should satisfy  $H_2^{in} > \lambda_{hy}$ , so that hydrogenotrophic bacteria can be maintained. Finally, the acetate inflow concentration should remain between the two Haldane thresholds  $\lambda_m^-$  and  $\lambda_m^+$ . In this range, methanogenic bacteria are maintained without going into the inhibitory phase. When  $S_{ac}^{in}$  or  $S_{ac}^*$  exceeds  $\lambda_m^+$ , bistable regions may appear. In these regions, high methane production is still possible, but low production may also exist. For this reason, the next step is to examine the basin of attraction in the bistable regions in order to identify the initial conditions leading to the stable equilibrium that yields the maximum methane production.



**Figure 10.** Worst methane production performance in the  $(S_{ac}^{in}, D)$  plane for  $S_s^{in} = 7.5$ ,  $S_{dc}^{in} = 1$  and  $H_2^{in} = 0.6$ . (a) Stable equilibrium yielding the lowest methane level. (b) Minimum methane concentration  $CH_4^e$ .

### 3.2.3. Basins of Attraction

The initial values of  $S_{ac}(0)$  and  $X_m(0)$  are varied, while the other initial conditions are given in Table 9. Figure 11 shows the basins of attraction in the bistable region  $R_{18}$  for the same inflow values used in Figure 10 and for two values of the dilution rate,  $D = 0.25$  and  $D = 0.3$ . In this region, we have  $S_{dc}^* > \lambda_a$  and  $H_2^{in} > \lambda_{hy}$ , so the acidogenic and hydrogenotrophic populations can be maintained. However, the acetate level is high, with  $S_{ac}^* > \lambda_m^+$ , which leads to bistability due to the Haldane inhibition effect. The two stable equilibria are  $(E_2, F_2)$  and  $(E_5, F_2)$ . The orange zone represents the initial conditions leading to  $(E_5, F_2)$ , while the blue zone represents the initial conditions leading to  $(E_2, F_2)$ . The unstable equilibria in this region do not attract an open set of initial conditions, but they contribute to the separation between the two basins of attraction. For  $D = 0.3$ , as shown in Figure 11a, the system is far from the favorable region  $R_{16}$ , and the basin of attraction of  $(E_2, F_2)$  becomes much larger. Thus, increasing the dilution rate reduces the basin of attraction of the favorable equilibrium  $(E_5, F_2)$ . In this case, a larger initial value of  $X_m(0)$  is required to avoid the washout of the methanogenic population in the AD step. This means that when the dilution rate is higher, a larger initial methanogenic biomass is needed to consume the acetate and reach the methane-producing equilibrium. For  $D = 0.25$ , as shown in Figure 11b, the system is close to the favorable region  $R_{16}$ . The basin of attraction of  $(E_5, F_2)$  occupies a large part of the plane  $(S_{ac}(0), X_m(0))$ . This means that the favorable equilibrium can be obtained with a reasonable initial value of methanogenic biomass. However, when  $X_m(0)$  is too small, the system converges to  $(E_2, F_2)$ , where the methanogenic bacteria of the AD step are washed out. The boundary between the two basins increases with  $S_{ac}(0)$ . This shows that a high initial acetate concentration does not necessarily improve methane production. Due to the Haldane inhibition effect, large values of  $S_{ac}(0)$ , combined with a large acetate inflow  $S_{ac}^{in}$ , require a larger initial methanogenic biomass to avoid convergence toward  $(E_2, F_2)$ . This suggests that the reactor should be initialized with a sufficient amount of methanogenic biomass and a low concentration of acetate, especially when the dilution rate is high.



**Figure 11.** Basins of attraction in the  $(S_{ac}(0), X_m(0))$  plane for the bistable region  $R_{18}$ , with  $S_s^{\text{in}} = 7.5$ ,  $S_{dc}^{\text{in}} = 1$ ,  $S_{ac}^{\text{in}} = 30$  and  $H_2^{\text{in}} = 0.6$ .

**Table 9.** Initial conditions.

| Initial Concentration | Value  |
|-----------------------|--------|
| $S_s(0)$              | 4      |
| $S_{dc}(0)$           | 1      |
| $X_a(0)$              | 0.1    |
| $H_2(0)$              | 0.002  |
| $X_{hy}(0)$           | 0.0015 |
| $p_{H_2}(0)$          | 0      |
| $CO_2(0)$             | 0      |
| $CH_4(0)$             | 0      |
| $p_{CO_2}(0)$         | 0      |
| $p_{CH_4}(0)$         | 0      |

#### 4. Discussion

We studied a coupled mathematical model for the methane production. It combines a three-stage anaerobic digestion process with hydrogenotrophic biomethanation and gas–liquid mass transfer. A qualitative analysis of the resulting dynamical system was performed, showing that the coupled model can exhibit up to twelve equilibria, whose local stability properties were studied in detail. The analytical study gives the explicit expressions for the equilibrium substrates  $S_{dc}^*$  and  $S_{ac}^*$  as functions of the influent substrates  $S_s^{\text{in}}$ ,  $S_{dc}^{\text{in}}$ , and  $S_{ac}^{\text{in}}$ , and the dilution rate  $D$ . It also gives the threshold values  $\lambda_a$ ,  $\lambda_m^-$ ,  $\lambda_m^+$ , and  $\lambda_{hy}$ , which determine the existence and stability regions of the equilibria. Based on these analytical conditions, the numerical simulations construct operating diagrams that show how the dilution rate and inflow concentrations can be selected to avoid washout and obtain favorable methane production.

From an engineering point of view, methane production through the AD pathway requires avoiding microbial washout and maintaining the activity of the acidogenic and methanogenic populations. The acidogenic population can be maintained when the dissolved biodegradable substrate is sufficiently available, that is, when  $S_{dc}^* > \lambda_a(D)$ . The methanogenic population is favored when the acetate concentration  $S_{ac}^*$  remains within the range delimited by the thresholds  $\lambda_m^-(D)$  and  $\lambda_m^+(D)$ . As shown in Figures 2 and 3, these threshold conditions divide the operating plane into regions with different asymptotic behaviors and indicate how the dilution rate and the inflow concentrations can be selected to reach favorable regions. In particular, the regions where the stable equilibrium  $E_5$  exists, such as region  $A_7$ , correspond to operating conditions where both acidogenesis and methanogenesis are maintained, leading to favorable methane production through the

AD pathway. This interpretation is consistent with previous studies based on operating diagrams for AD models [8,9].

The coupled biomethanation pathway provides additional operating guidelines related to hydrogen availability. When  $H_2^{\text{in}} = 0$ , or more generally, when  $H_2^{\text{in}} \leq \lambda_{hy}(D)$ , the hydrogenotrophic biomass cannot be maintained and the contribution of biomethanation is not supported. In this case, methane production is mainly driven by the AD pathway. As illustrated in Figures 5 and 8, the favorable operating region is then associated with the stable equilibrium  $(E_5, F_1)$ , such as in region  $R_7$ , where  $E_5$  maintains methane production through AD, while  $F_1$  corresponds to the elimination of the hydrogenotrophic population. In contrast, when  $H_2^{\text{in}} > \lambda_{hy}(D)$ , the hydrogenotrophic population can contribute to the conversion of carbon dioxide to methane. In this situation, increasing the hydrogen inflow can enhance methane production, provided that AD populations are also maintained. The operating diagrams show that the favorable coupled region is then associated with the stable equilibrium  $(E_5, F_2)$ , such as in region  $R_{16}$ . This equilibrium combines methane production from the AD pathway with additional methane production through hydrogenotrophic biomethanation.

When the inflow acetate concentration level exceeds the upper threshold  $\lambda_m^+$ , bistability may occur. In this case, high methane production is still possible, but it depends on the initial state of the reactor. In particular, a sufficient initial methanogenic biomass may be needed to drive the system towards the high methane-producing equilibrium. The basin of attraction analysis also shows that for lower dilution rates, the basin of favorable equilibrium becomes larger. Therefore, high methane production can be reached for a larger set of initial conditions. Thus, in bistable regions, operating strategies should not only aim to maximize methane production but also to drive the reactor state toward the basin of attraction of the desired equilibrium.

Future work will primarily focus on the bistability issue identified in this study. Based on the operating diagrams and methane production maps obtained, a first direction will be to develop a more complete analytical description of the operating regions where methane production is maximal and associated with a stable favorable equilibrium. The second direction will be to extend the present operating diagram analysis with a more detailed bifurcation study with respect to the dilution rate and the main inflow concentrations. This would allow for a more precise description of the transitions between the stable, bistable, and washout regimes. In bistable regions, a more detailed study of the attraction basins will be considered to identify the initial microbial concentrations that drive the reactor to a state of high methane production.

The present work should be interpreted as a purely theoretical and numerical study, and the model predictions require subsequent experimental verification. However, the operating diagrams indicate how the dilution rate and the inflow concentrations can be chosen in order to favor methane production through AD and hydrogenotrophic biomethanation. The model should be used under the assumptions made in its formulation. In particular, Hypothesis (H4) assumes that carbon dioxide remains sufficiently available, so that hydrogen is the main limiting substrate for hydrogenotrophic biomethanation. Under this assumption, the hydrogenotrophic growth rate can be described by a single-substrate Monod function depending only on dissolved hydrogen. However, if carbon dioxide becomes limiting, a dual-substrate limitation function depending on both dissolved hydrogen and dissolved carbon dioxide should be considered.

Finally, selected model extensions, such as hydrogen inhibition under excess hydrogen conditions, possible hydrogen production within the AD pathway, and spatial effects, should be investigated in future work to determine their effects on the stability regions, bistability structure, and methane production outcomes.

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## Abbreviations

The following abbreviations are used in this manuscript:

|      |                                |
|------|--------------------------------|
| AD   | Anaerobic digestion            |
| ADM1 | Anaerobic digestion model No 1 |
| AM2  | Anaerobic model No 2           |
| VFA  | Volatile fatty acid            |
| S    | Stable                         |
| U    | Unstable                       |

## Appendix A. Analysis of Asymptotic Behavior

### Appendix A.1. Proof of Proposition 1

- Component  $S_s$ . We have

$$\frac{dS_s}{dt} = -k_h S_s + D(S_s^{\text{in}} - S_s), \quad S_s(0) = S_s^0.$$

Since  $D S_s^{\text{in}} \geq 0$ , we have  $\frac{dS_s}{dt} \geq -(k_h + D)S_s$ . By the comparison principle [25], if  $S_s^0 > \underline{S}^0$ , then  $S_s(t) \geq \underline{S}(t)$ , where  $\underline{S}$  satisfies

$$\frac{d\underline{S}}{dt} = -(k_h + D)\underline{S}, \quad \underline{S}(0) = \underline{S}^0. \quad (\text{A1})$$

If  $\underline{S}^0 = 0$ , then  $\underline{S} \equiv 0$  is the unique solution of Equation (A1). Hence,  $S_s(t) \geq 0$  for all  $t \geq 0$ , and  $S_s(t) > 0$  whenever  $S_s^0 > 0$ .

- Component  $X_a$ . The equation for  $X_a$  is

$$\frac{dX_a}{dt} = (\mu_a(S_{dc}) - D)X_a, \quad X_a(0) = X_a^0.$$

This equation is linear with respect to  $X_a$ . Therefore, for any  $X_a^0 \geq 0$ , we obtain  $X_a(t) \geq 0$  for all  $t \geq 0$ .

Since Subsystem (1) has a cascade structure, the same reasoning applies to the remaining components. The positivity of  $S_{dc}$  and  $S_{ac}$  follows from the comparison principle as in the case of  $S_s$ , while the variables  $X_m$  and  $X_{hy}$  satisfy linear equations and therefore remain non-negative as long as their initial concentrations are non-negative.

- Components  $H_2$ ,  $p_{H_2}$ ,  $p_{CO_2}$ , and  $p_{CH_4}$ . The equations describing  $H_2$ ,  $p_{H_2}$ ,  $p_{CO_2}$ , and  $p_{CH_4}$  have the same structure, and their reaction terms satisfy the quasi-positivity property. Denoting by  $f_i$  the right-hand side of the equation

$$\frac{du_i}{dt} = f_i(u_1, \dots, u_n), \quad i = 1, \dots, n$$

we have

$$f_i(u_1, \dots, u_{i-1}, 0, u_{i+1}, \dots, u_n) \geq 0 \quad \text{for all } u_j \geq 0, j \neq i.$$

Therefore, the non-negative orthant is invariant, and for non-negative initial concentrations, we have

$$H_2(t) \geq 0, \quad p_{H_2}(t) \geq 0, \quad p_{CO_2}(t) \geq 0, \quad p_{CH_4}(t) \geq 0, \quad \text{for all } t \geq 0.$$

- Component  $CO_2$ . Under hypothesis (H4), the right-hand side of the  $CO_2$  equation, denoted  $f_{CO_2}$ , satisfies the quasi-positivity property, namely

$$f_{CO_2}(\dots, 0, \dots) \geq 0 \quad \text{when all other components are non-negative.}$$

Then, for any  $CO_2^0 \geq 0$ , we obtain  $CO_2(t) \geq 0$  for all  $t \geq 0$ .

- Component  $CH_4$ . We assume that (see Remark A1 for the biological interpretation)

$$\mu_{hy}(H_2) - m \geq 0. \quad (A2)$$

Under condition (A2), the right-hand side of equation  $CH_4$ , denoted  $f_{CH_4}$ , satisfies the quasi-positivity property, namely

$$f_{CH_4}(\dots, 0, \dots) \geq 0 \quad \text{when all other components are non-negative.}$$

Then, for any  $CH_4^0 \geq 0$ , we obtain  $CH_4(t) \geq 0$  for all  $t \geq 0$ .

#### Appendix A.2. Proof of Proposition 2

For Subsystem (1), consider the function

$$V_1(t) = y_{ac}(S_s(t) + S_{dc}(t) + X_a(t)) + \frac{1}{y_m}X_m(t) + S_{ac}(t).$$

We have

$$\frac{dV_1}{dt} = c - DV_1(t),$$

with

$$c = D(S_{ac}^{\text{in}} + y_{ac}S_{dc}^{\text{in}} + y_{ac}S_s^{\text{in}}).$$

By Gronwall's lemma,

$$0 \leq V_1(t) \leq \max\left\{V_1(0), \frac{c}{D}\right\} \quad \text{for all } t \geq 0.$$

Therefore,  $S_s$ ,  $S_{dc}$ ,  $X_a$ ,  $S_{ac}$  and  $X_m$  are bounded.

For Subsystem (2), let

$$k = k_{L,H_2}, \quad h = h_{H_2}, \quad \alpha = \frac{v_l}{v_g}rT.$$

Consider the function

$$V_2(t) = H_2(t) + \frac{1}{y_{X_{hy}}}X_{hy}(t) + \beta p_{H_2}(t),$$

where

$$\beta = \frac{1}{\alpha} \left(1 + \frac{D}{2k}\right).$$

We have

$$\frac{dV_2}{dt} = DH_2^{\text{in}} - \frac{D}{2}H_2 - \left(m + \frac{D}{y_{X_{hy}}}\right)X_{hy} - \frac{D}{2h}p_{H_2}.$$

Hence,

$$\frac{dV_2}{dt} \leq DH_2^{\text{in}} - \gamma V_2(t),$$

where

$$\gamma = \min\left\{\frac{D}{2}, y_{X_{hy}}\left(m + \frac{D}{y_{X_{hy}}}\right), \frac{D}{2h\beta}\right\} > 0.$$

By Gronwall's lemma,

$$0 \leq V_2(t) \leq \max\left\{V_2(0), \frac{DH_2^{\text{in}}}{\gamma}\right\} \quad \text{for all } t \geq 0.$$

Therefore,  $H_2$ ,  $X_{hy}$  and  $p_{H_2}$  are bounded.

For Subsystem (3), let

$$k_1 = k_{L,CO_2}, \quad k_3 = k_{L,CH_4}, \quad \alpha = \frac{v_1}{v_g}rT.$$

Consider the function

$$V_3(t) = CO_2(t) + CH_4(t) + \beta_1 p_{CO_2}(t) + \beta_2 p_{CH_4}(t),$$

where

$$\beta_1 = \frac{1}{\alpha}\left(1 + \frac{D}{2k_1}\right), \quad \beta_2 = \frac{1}{\alpha}\left(1 + \frac{D}{2k_3}\right).$$

We have

$$\frac{dV_3}{dt} = DCO_2^{\text{in}} + g(t) - k_{CO_2}(\mu_{hy}(H_2) - m)X_{hy} - \frac{D}{2}CO_2 - \frac{D}{2}CH_4 - \frac{D}{2h_{CO_2}}p_{CO_2} - \frac{D}{2h_{CH_4}}p_{CH_4},$$

where

$$g(t) = (1 - y_{ac})\left(\frac{1 - y_a}{y_a}\mu_a(S_{dc})\right)X_a + y_{CO_2}\left(\frac{1 - y_m}{y_m}\mu_m(S_{ac})\right)X_m + k_{CH_4}(\mu_{hy}(H_2) - m)X_{hy}.$$

Since  $(\mu_{hy}(H_2) - m) \geq 0$  and the solutions of the two subsystems are bounded, there exists  $g^{\max} > 0$  such that

$$g(t) \leq g^{\max} \quad \text{for all } t \geq 0.$$

Hence,

$$\frac{dV_3}{dt} \leq DCO_2^{\text{in}} + g^{\max} - \gamma_1 V_3(t),$$

where

$$\gamma_1 = \min\left\{\frac{D}{2}, \frac{D}{2h_{CO_2}\beta_1}, \frac{D}{2h_{CH_4}\beta_2}\right\} > 0.$$

By Gronwall's lemma,

$$0 \leq V_3(t) \leq \max\left\{V_3(0), \frac{DCO_2^{\text{in}} + g^{\max}}{\gamma_1}\right\} \quad \text{for all } t \geq 0.$$

Therefore,  $CO_2$ ,  $CH_4$ ,  $p_{CO_2}$  and  $p_{CH_4}$  are bounded.

**Remark A1.** Condition (A2) means that the hydrogen concentration is sufficient to cover the maintenance demands of the hydrogentrophic biomass, so that methane production remains non-negative. If condition (A2) is not satisfied, several models in the literature replace the term with its positive part [22]

$$(\mu_{hy}(H_2) - m)^+ = \max\{\mu_{hy}(H_2) - m, 0\},$$

which avoids negative methane production rates.

#### Appendix A.3. Proof of Proposition 4

At  $E_5$ , we have  $X_a^* > 0$  and  $X_m^{*-} > 0$ ; hence, the four remaining eigenvalues of the Jacobian matrix are the solutions of the characteristic Equations (10) and (13), with  $(S_{dc}, X_a) = (\lambda_a, X_a^*)$  and  $(S_{ac}, X_m) = (\lambda_m^-, X_m^{*-})$ . For the Monod function,  $\mu'_a(\lambda_a) > 0$ , and therefore

$$\text{tr}(A(E_5)) = -D - \frac{1}{y_a} \mu'_a(\lambda_a) X_a^* < 0, \quad \det(A(E_5)) = D \frac{1}{y_a} \mu'_a(\lambda_a) X_a^* > 0.$$

For the Haldane function, the solution  $\lambda_m^-$  is on the increasing branch; hence,  $\mu'_m(\lambda_m^-) > 0$ , and similarly,

$$\text{tr}(B(E_5)) = -D - \frac{1}{y_m} \mu'_m(\lambda_m^-) X_m^{*-} < 0, \quad \det(B(E_5)) = D \frac{1}{y_m} \mu'_m(\lambda_m^-) X_m^{*-} > 0.$$

Thus, both eigenvalues of  $A(E_5)$  and of  $B(E_5)$  have negative real parts. Since  $-(k_h + D) < 0$  is an additional eigenvalue,  $E_5$  is locally asymptotically stable.

#### Appendix A.4. Proof of Proposition 5

At  $E_4$  and  $E_6$ , we have  $S_{ac} = \lambda_m^+$  and  $X_m > 0$ . For the Haldane function,  $\lambda_m^+$  is on the decreasing branch; hence,  $\mu'_m(\lambda_m^+) < 0$ . Therefore, from Equation (14),

$$\det(B(E_4)) = D \frac{1}{y_m} \mu'_m(\lambda_m^+) X_m^{*+} < 0, \quad \det(B(E_6)) = D \frac{1}{y_m} \mu'_m(\lambda_m^+) X_m^{*+} < 0.$$

Since  $B$  is a real  $2 \times 2$  matrix, its eigenvalues  $\lambda_4, \lambda_5$  satisfy  $\lambda_4 \lambda_5 = \det(B)$ . If  $\det(B) < 0$ , then  $\lambda_4 \lambda_5 < 0$ , so  $\lambda_4$  and  $\lambda_5$  have opposite signs; in particular,  $B$  has a positive eigenvalue. Therefore, the Jacobian has a positive eigenvalue and the equilibrium is unstable.

#### Appendix A.5. Proof of Proposition 6

Recall that  $\lambda_1 = -(k_h + D) < 0$  is an eigenvalue of the Jacobian.

1. Stability of  $E_1$ . At  $E_1$ , we have  $X_a = X_m = 0$ . Hence, by Equations (9) and (12), the remaining eigenvalues are  $-D$ ,  $\mu_a(S_{dc}^*) - D$ ,  $-D$ , and  $\mu_m(S_{ac}^{\text{in}}) - D$ . Therefore, all eigenvalues are negative if and only if  $\mu_a(S_{dc}^*) < D$  and  $\mu_m(S_{ac}^{\text{in}}) < D$ .
2. Stability of  $E_2$ . At  $E_2$ , we have  $X_m = 0$ , so block  $B$  adds the eigenvalues  $-D$  and  $\mu_m(S_{ac}^*) - D$  by Equation (12). Moreover,  $(S_{dc}, X_a) = (\lambda_a, X_a^*)$  with  $X_a^* > 0$  and  $\mu_a(\lambda_a) = D$ . Using Equation (11),

$$\text{tr}(A(E_2)) = -D - \frac{1}{y_a} \mu'_a(\lambda_a) X_a^* < 0, \quad \det(A(E_2)) = D \frac{1}{y_a} \mu'_a(\lambda_a) X_a^* > 0.$$

Hence, the two eigenvalues of  $A(E_2)$  have negative real parts. Then,  $E_2$  is stable if and only if  $\mu_m(S_{ac}^*) - D < 0$ , i.e.,  $\mu_m(S_{ac}^*) < D$ .

3. Stability of  $E_3$ . At  $E_3$ , we have  $X_a = 0$ , so block  $A$  adds the eigenvalues  $-D$  and  $\mu_a(S_{dc}^*) - D$  by Equation (9). Furthermore,  $(S_{ac}, X_m) = (\lambda_m^-, X_m^{*-})$  with  $X_m^{*-} > 0$  and  $\mu_m(\lambda_m^-) = D$ . Since  $\lambda_m^-$  is on the increasing branch of the Haldane function,  $\mu'_m(\lambda_m^-) > 0$ , and Equation (14) yields

$$\text{tr}(B(E_3)) = -D - \frac{1}{y_m} \mu'_m(\lambda_m^-) X_m^{*-} < 0, \quad \det(B(E_3)) = D \frac{1}{y_m} \mu'_m(\lambda_m^-) X_m^{*-} > 0,$$

so the two eigenvalues of  $B(E_3)$  have negative real parts. Hence,  $E_3$  is stable if and only if  $\mu_a(S_{dc}^*) < D$ .

#### Appendix A.6. Proof of Proposition 8

At  $F_2$ , we have  $\mu_{hy}(\lambda_{hy}) = a$  and

$$y_{X_{hy}}(\mu_{hy}(\lambda_{hy}) - m) - D = 0, \quad X_{hy}^* > 0, \quad \mu'_{hy}(\lambda_{hy}) > 0.$$

Let  $\chi(\lambda) = \lambda^3 + c_1\lambda^2 + c_2\lambda + c_3$  denote the characteristic equation of  $J(F_2)$  with

$$c_1 = (D + k + s) + \frac{\alpha k}{h}, \quad c_2 = \frac{\alpha k}{h}(D + s) + a y_{X_{hy}} s, \quad c_3 = \frac{\alpha k}{h} a y_{X_{hy}} s,$$

where  $s = \mu'_{hy}(\lambda_{hy}) X_{hy}^* > 0$ . Hence,  $c_1 > 0$ ,  $c_2 > 0$ ,  $c_3 > 0$ , and moreover

$$c_1 c_2 - c_3 = \frac{\alpha k}{h}(D + s) \left( (D + k + s) + \frac{\alpha k}{h} \right) + a y_{X_{hy}} s (D + k + s) > 0.$$

Therefore, by the Routh–Hurwitz criteria for cubic polynomials, all solutions of  $\chi$  have negative real parts, and  $F_2$  is locally asymptotically stable.

#### Appendix A.7. Proof of Proposition 9

At  $F_1$ , we have  $X_{hy} = 0$ . Then, one eigenvalue is

$$\lambda_6 = y_{X_{hy}}(\mu_{hy}(H_2^{\text{in}}) - m) - D.$$

Hence,  $\lambda_6 < 0$  if and only if  $\mu_{hy}(H_2^{\text{in}}) < a$ . The two remaining eigenvalues are those of the matrix  $C$ , which satisfies  $\text{tr}(C) = -(D + k) - \frac{\alpha k}{h} < 0$  and  $\det(C) = \frac{\alpha k D}{h} > 0$ . Therefore, both eigenvalues of  $C$  have negative real parts. We conclude that  $F_1$  is locally asymptotically stable if and only if  $\mu_{hy}(H_2^{\text{in}}) < a$ .

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