Reliable determination of the growth and hydrogen production parameters of the photosynthetic bacterium *Rhodobacter capsulatus* in fed batch culture using a combination of the Gompertz function and the Luedeking-Piret model

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ABSTRACT

In this study, experimental results of hydrogen producing process based on anaerobic photosynthesis using the purple non-sulfur bacterium *Rhodobacter capsulatus* are scrutinized. The bacterial culture was carried out in a photo-bioreactor operated in a quasi-continuous mode, using lactate as a carbon source. The method is based on the continuous stirred tank reactors (CSTR) technique to access kinetic parameters. The dynamic evolution of hydrogen production as a function of time was accurately simulated using Luedeking-Piret model and the growth of *R. capsulatus* was computed using Gompertz model. The combination of both models was successfully applied to determine the relevant parameters (*λ, μ* max, α and β) for two *R. capsulatus* strains studied: the wild-type strain B10 and the H₂ over-producing mutant IR3. The mathematical description indicates that the photofermentation is more promising than dark fermentation for the conversion of organic substrates into biogas.

1. Introduction

Economic development over the last few decades has been strongly dependent on fossil fuels as sources of energy. These resources are not unlimited in the long term, and environmental concerns have led to the search for clean, renewable energy sources. Urban/Agro-industrial/ agricultural wastes appear as relevant energetic resources in a sustainable energy mix. Biogas results from the anaerobic digestion of organic matter that is, the main constituent of these wastes. Indeed, the production of methane or hydrogen from the biomass follows the principle of the fermentation. Hydrogen is considered as the more sustainable energy carrier due to the high efficient end-use technologies such as the fuel cells [1, 2]. Clean-hydrogen gas can be produced either by electrolysis [3], steam biomethane reforming [4], biomass gasification [5], or biological synthesis [6, 7].

Biohydrogen production processes, are divided into two groups according to the dependency on light: dark and photo fermentations [8, 9]. Under dark anaerobic conditions, the organic substrates and waste waters are metabolized to form hydrogen and lower molecular weight organic acids. Photofermentative hydrogen production, issued from oxidation of organic compounds, occurs under anaerobic, nitrogen-limited conditions, utilizing light as energy source. A wide range of photosynthetic bacteria has been reported to produce hydrogen, including *Rhodobacter capsulatus, Rhodobacter sphaeroides, Rhodopseudomonas palustris,* and *Rhodospirillum rubrum* [10, 11]. Among them, *Rhodobacter capsulatus* is a favorable candidate for large-scale production due to its high energy and substrate conversion efficiencies and its ability to utilize a wide variety of substrates for growth and hydrogen production [12, 13].

The rate and yield of hydrogen production is greatly dependent on the carbon source used, physiological growth conditions, such as light intensity [14, 15], and bacterial growth mode. Fed-batch growth mode is recognized as the most suitable operation mode for H₂ production, in comparison with batch and continuous modes [16]. In fact, studies for H₂ production during fed-batch conditions by photosynthetic bacteria have been reported for *Rhodopseudomonas palustris* sp. and *R. palustris* 420L (on acetate and malate as carbon source, respectively) [17, 18].
Various kinetics models have been derived for biohydrogen production, as previously reviewed [21, 22]. In particular, and because of its simple initial form, the Luedeking-Piret model, developed in 1959 to describe lactic acid production and others processes [23, 24], has recently been applied to fermentative hydrogen production. In this widely used mathematical model, the rate of product formation (like hydrogen) can be related to both biomass concentration and microbial growth rate. All the studies concerned dark fermentation conditions with the exception of one devoted to the phototrophic bacterium, _R. palustris_ [25].

Unfortunately, comparison between studies is quite complicated because the use of batch culture and different operating conditions makes it difficult to determine Luedeking-Piret model parameters from the published data. The estimation of not directly quantifiable compounds is essential in the development of biotechnology processes like photo-hydrogen production. However, a complex model involves several kinetics parameters and it is difficult to discuss the relevance of each one. It is also difficult to use traditional batch techniques, without complex model, to scrutinize useful parameters. This problem is theoretically overcome with continuous stirred tank reactors (CSTR) inside which the composition is uniform at any point. For a given mean residence time (\(\overline{t}\)) and inlet and outlet concentrations, the mass balance is very simple and it is hence easy to calculate the kinetic constants. In practice, the stirring of bioreactor is well achieved with a good mixing but the inlet flow rate must be adapted to avoid wash-out of microbial biomass.

The present study describes a mathematical approach to determine reliable kinetic parameters of hydrogen production by the photosynthetic bacterium, _R. capsulatus_, in a quasi-continuous photo-bioreactor. The developed mathematical/experimental approach is simple enough in terms of mathematical complexity and experimental procedure to be further used to attain reliable parameter values (\(\alpha, \beta\)) of Luedeking-Piret model or maximum growth rate (\(\mu_{\text{max}}\)) and lag time (\(\lambda\)). The present work combines Luedeking-Piret model and Gompertz model using the main assumptions of continuous stirred-tank reactor (CSTR) operation.

2. Material and methods

2.1. Bacteria and culture medium

_Rhodobacter capsulatus_ strains B10 and IR3 [26] were grown anaerobically at pH 6.8 and 30 °C in modified RCV medium. The modified medium RCV, derived from [27], contained Na-lactate (35 mM), Na-Glutamate (7 mM), phosphate buffer (K<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub>, 5.17 and 4.41, mM), and a solution of salts containing different salts as oligoelements such as Mo (1.5μmol) and Fe (42 μmol).

2.2. Photo-bioreactor

Hydrogen producing experiments were carried out in a laboratory-made, rectangular-shaped 1 L bioreactor operated in the fed batch mode [28]. Anaerobic conditions were obtained by initially purging the bioreactor with sterile argon gas. Temperature and pH values were acquired by a thermocouple and pH probe via a computer via an acquisition card. The reactor was illuminated from one side by a sodium-vapor lamp (OSRAM, Plantastar, 600 W). The light intensity at the surface of the reactor was varied by changing the distance between the light source and the illuminated surface. The light intensity was measured with a digital lux meter (Meter, RO 1332).

The dynamics of the bacterial population and the hydrogen production were measured in quasi-continuous mode. The culture was started in batch mode, then when a constant bacterial protein concentration was reached ca. 0.45 g/L for B10 and ca. 0.36 g/L for IR3. As soon as the bacterial concentration was reached, the quasi-continuous conditions were applied. During continuous operation, the culture was fed with RCV growth medium containing 35.7 mM (4 g/L) sodium lactate and 5 mM sodium glutamate at a rate of 1 ml min<sup>-1</sup> and bacterial culture was withdrawn at the same rate over a 30–50 h period. The withdrawal and feeding were then interrupted and the culture operated in batch mode for 6 h. These quasi-continuous conditions were repeated 3–5 times period of 100–200 h for both _R. capsulatus_ strains. In the case of strain IR3, the final batch mode was prolonged until stationary phase was achieved.

2.3. Analytical methods

Samples were regularly withdrawn for determining the concentrations of biomass and substrate. The bacterial cell concentration was estimated by measuring the optical density at 660 nm according to the relation OD 0.8 corresponded to 0.37 mg dry weight L<sup>-1</sup> and 0.16 mg protein L<sup>-1</sup>. Lactate concentration was measured by HPLC (Agilent 1260, column Hi Flex H 8μm) using a Refractive Index Detector G1362A – Agilent. Samples were firstly centrifuged (16,060 g, 5 min) and the supernatant was diluted 10 times and finally filtered (0.2 μm porosity) before analysis. The mobile phase for HPLC was a solution of sulfuric acid 2.3 mM (0.4 ml min<sup>-1</sup>). Calibration curves were generated using standards of ultrapure lactic acid.

2.4. Mathematical approach

The Gompertz function, available since 1825, can be used as exponential model to take into account the slowing down of the observed growth of the batch reactor culture of any microorganism. The Gompertz function can describe the dynamics of any living form without limitation of resources. Consequently, the Gompertz function [29] is well adapted to biological reactors with efficient substrate renewal.

The Gompertz equation is given by:

\[
\ln(\frac{X}{X_0}) = ae^{-\beta t}
\]  

(1)

We call this relationship \(f(t)\) and the associated equation is

\[
g(t) = X_0e^{-\frac{\beta t}{\lambda}}
\]  

(2)

where \(X\) is the bacterial density or protein concentration and \(X_0\) is the initial bacterial density or initial protein concentration. First and second derivatives of \(f(t)\) are given by the equation (3) and equation (4):

\[
f'(t) = aee^{-\beta(t-\lambda)}
\]  

(3)

\[
f''(t) = aee^{-\beta(t-\lambda)} - a\beta e^{-\beta(t-\lambda)}(e^{\beta\lambda} - 1)
\]  

(4)

Gibson et al. [29] have coupled logistic models and the Gompertz function; however the Gompertz function is not directly interpretable. To solve this problem, Zwieten et al. [30] simplified the two models by incorporating the classic parameters of maximum concentration, latency time and maximum growth rate. However, it is also possible to determine these parameters using the nonphysical parameters (raw parameters) of
the Gompertz function (a, b, c) using the undermentioned equations. The time of inflexion point is the solution of the following equation:

\[ f'(t_i) = 0 \]  

(5)

Which involves:

\[ t_i = \frac{b}{c} \]  

(6)

And the maximum growth (\( \mu_{\text{max}} \)) rate is given by:

\[ \mu_{\text{max}} = \lim_{t \to +\infty} f(t) \]  

(7)

The lag time (\( t_\text{l} \)) is the intercept with x-axis of the tangent line:

\[ f(t_i) + y(t_i) = 0 \]  

(8)

And the maximum concentration is the asymptote of Gompertz function:

\[ X = \lim_{t \to +\infty} X_0 e^{1 - e^{-ct}} = X_0 \lim_{t \to +\infty} e^{1 - e^{-ct}} \]  

(9)

Overall techniques are well known, but few experimental cultures correspond to the simple sigmoid function (Gompertz function). In other hand, Luedeking-Piret model can well describes the fermentative process; this classical model considers the relationship of cell growth to substrate limitation (stabilization at 0.7 g L\(^{-1}\)) during the continuous mode operation was computed to be close to 20 mg of proteins per hour for an average flow rate < 1 ml min\(^{-1}\). This is small compared to the potential range of biomass production in the reactor of 100–350 mg protein per hour. Taken together with the lack of substrate limitation (stabilization at 0.7–0.95 g lactate L\(^{-1}\)), these results validate the assumptions of the Gompertz model.

### 3.2. Fitting procedure and parametric values

The aim of the “data fitting” is to determine and compare parameter values of the model that describes the process. On the other hand, the parametric optimization is also a useful tool for validating the model.

![Figure 1. Kinetics of the lactate consumption (S, ◊) and the bacterial production (X, ▲) during a quasi-continuous culture of R. capsulatus B10 (A) and IR3 (B). The arrows indicate the application of continuous flow.](image)

For the wild type B10 strain, the first feeding procedure started at the beginning of the batch stationary phase (Figure 1A), when lactate was almost completely metabolized (concentration close to 0 g L\(^{-1}\)). The application of continuous flow conditions led to an increase in lactate concentration to 0.7 g L\(^{-1}\), concomitant with a decrease in bacterial protein concentration from 0.54 to 0.44 g L\(^{-1}\). Interruption of continuous flow followed by batch culture then led to a stabilization at 0.7–0.8 g lactate L\(^{-1}\) and 0.44–0.48 g protein L\(^{-1}\). This was repeated over 3 cycles of quasi-continuous operation.

For the H\(_2\) over-producing strain IR3, the feeding procedure started at the end of the exponential growth phase, after the consumption of 90% of lactate (residual concentration of 0.4 g L\(^{-1}\)). The feeding of growth medium led to an increase in lactate concentration to 1 g L\(^{-1}\) and a decrease in bacterial protein concentration from 0.4 g L\(^{-1}\) to 0.35 g L\(^{-1}\). Alternation of continuous and batch modes led to a stabilization at 0.34–0.38 g protein L\(^{-1}\) and 0.75–0.95 g lactate L\(^{-1}\), and this was repeated over 5 cycles of quasi-continuous operation. The final, prolonged batch phase led to complete consumption of lactate and a final bacterial concentration of 0.45 g L\(^{-1}\).

In both case the decrease in bacterial concentration due to wash-out during the continuous mode operation was computed to be close to 20 mg of proteins per hour for an average flow rate < 1 ml min\(^{-1}\). This is small compared to the potential range of biomass production in the reactor of 100–350 mg protein per hour. Taken together with the lack of substrate limitation (stabilization at 0.7–0.95 g lactate L\(^{-1}\)), these results validate the assumptions of the Gompertz model.

### 3. Results and discussion

#### 3.1. Experimental measurements of bacterial growth and substrate consumption

Kinetics of bacterial production and substrate consumption for the wild type R. capsulatus B10 and the H\(_2\) over-producing strain IR3 were shown in Figures 1A-1B, respectively.
Nevertheless, the fitting procedure requires an appropriate attention to achieve relevant results. The Gradient methods are generally more efficient when the objective function is continuous in its first derivative. Gradient methods use information about the slope of the function to dictate a direction of search where the minimum is thought to lie. The simplest of these is the method of steepest descent in which a search is performed in an opposite direction of gradient of the objective function. This method is available with numerous commercial software. Gompertz function and (Eq. 1) coupled Luedeking-Piret model and Gompertz function (Eq. 12) are expedient mathematical functions to achieve the conventional numerical optimizations.

Figure 2. Bacterial growths in quasi-continuous culture of wild strain B10 (●) and over-producer strain IR3 (○): experimental data and Gompertz simulation.

Bacterial growth was modeled using the Gompertz function; and Figure 2 shows good agreement between the model and experimental values of biomass concentration. Table 1 compares the a, b and c values for the R. capsulatus strains. However, no direct interpretation is possible as these are raw parameters, and the Eqs. (5), (6), (7), and (8) must be used in order to obtain meaningful physical parameters, such as $\mu_{\text{max}}$, $\lambda$, and $Y_{xS}$.

In this context, $\lambda$ is the intercept with x-axis of the tangent line as expressed in the Eq. (7), $\mu_{\text{max}}$ is given by the derivative function of Gompertz model (Eq. (7)) and $Y_{xS}$ is directly obtained from Eq. (9) and the initial substrate concentration.

Figure 3. Simulated Luedeking-Piret’s model coupled Gompertz equation to and H$_2$-production by Rhodobacter capsulatus B10 (A) and IR3 (B) (35 mM lactate, 30,000 Lx).
The growth-associated product coefficient, α, and the non-growth-associated product coefficient, β, were determined by numerical optimization from experimental data and the Luedeking-Piret model Eq. (12) (i.e. data fitting). Experimental data for H₂ production were correctly modelled for both strains B10 and IR3, as shown in Figure 3, and the fitted parameters are summarized in Table 2.

The corresponding physical parameters, μ<sub>max</sub>, Y<sub>XS</sub>, λ, α, β were summarized in Table 2.

| μ<sub>max</sub> [h⁻¹] | Y<sub>XS</sub> | λ [h⁻¹] | α [ml H₂ (g L⁻¹)⁻¹ h⁻¹] | β [ml H₂ (g L⁻¹)⁻¹ h⁻¹] |
|---------------------|------------|---------|------------------------|------------------------|
| B10                 | IR3        | B10     | IR3                    | B10                    |
| 9.8                 | 13.9       | 0.195   | 0.166                  |                      |
| 4.5                 | 36.6       | 0.9980  | 0.9975                 |                      |
| 0.9942              | 0.9969     | 0.74    | 0.53                   |                      |

The Luedeking-Piret model implies that hydrogen production is associated with both non-growth and growth-associated terms. The growth-associated term indicates that hydrogen production (α) is proportional to the bacterial growth rate. On the other hand, the non-growth associated term (β) signifies that hydrogen production is linearly dependent on biomass concentration. In the case of strain IR3, both parameters were substantially higher than for the wild type strain B10 (see Table 2). It is noteworthy that the β parameter is main parameter to describe hydrogen production by photofermentation. Eq. (13) shows that it possible to model fermentation products using a linear relationship, assuming a negligible artificial mortality and biomass dilution during the continuous or quasi-continuous process:

\[
P(t) = b + \frac{\beta X(t)}{\mu_X (t) + \alpha_X (t)}
\]

Therefore, the non-growth associated term could be attained by direct linear regression and the parameters determined by the linear procedure (equation 13) were very close to those obtained by the overall simulation of the Luedeking–Piret model. Both mathematical procedures can provide realistic β parameters, which is the most important parameter when selecting a productive strain. One cannot estimate α separately using batch culture data (Eq. (12)), except for the parameters and concomitant α and β fitting is complicated by the necessary approximations of growth. Thus, in the case of batch cultures, realistic estimates of β and α require much additional data.

### 3.3. Discussion

Using the Gompertz function to model bacterial growth and the Luedeking-Piret model to describe hydrogen production by *R. capsulatus* during quasi-continuous culture, good agreement was observed between the experimental data and the models, with high regression coefficient values (R²) exceeding 0.99 in all cases (Table 2). The β and α parameters were lower in the wild type strain B10 than in the H₂ over-producer mutant IR3, as previously observed in batch culture [28]. The authors showed that the specific hydrogen production rate (ml h⁻¹) is proportional to the light intensity, which is the multiplying factor applied to non-growth-associated term (β) of Luedeking-Piret model. As above, in the present study, the wild type B10 exhibited a maximum specific growth rate, μ<sub>max</sub>, higher (0.195 h⁻¹) than the over-producer strain IR3 (0.166 h⁻¹), this latter converted more efficiently the substrate (lactate) in product (H₂) that the wild-type B10 strain.

The maximum growth rate observed in indoor culture on synthetic medium with the wild-type *R. capsulatus* B10, on lactate-glutamate (35/5 mM as carbon and nitrogen source, is comparable with this obtained with the strain 37b4 on malate-(NH₄)₂SO₄ (16/9.5 mM), 0.195 and 0.251 h⁻¹ [32]. However, much lower maximum growth rates were observed in outdoor fed batch cultures on acetate-glutamate medium of *R. capsulatus* DSM1710 and the hydrogenase-deficient mutant YO3 (0.025 h⁻¹ and 0.052 h⁻¹, respectively [13, 33]. By contrast, in the present study both strains (IR3 and B10) exhibit similar values of the conversion rate of

### Table 1. Gompertz function parameters.

|        | B10  | IR3  |
|--------|------|------|
| α [-]  | 3.03 | 2.66 |
| b [-]  | 1.77 | 2.18 |
| c [h⁻¹]| 0.174| 0.1581|
| R²     | 0.9924| 0.9968|

### Table 2. Fitted parameters of Luedeking-Piret's model and associated growth parameters.

|        | B10  | IR3  |
|--------|------|------|
| Y<sub>XS</sub> | 0.124 | 0.109 |
| λ [h⁻¹]  | 9.8  | 13.9 |
| μ<sub>max</sub> [h⁻¹] | 0.195 | 0.166 |
| β [ml H₂ (g L⁻¹)⁻¹ h⁻¹] | 17 | 72 |
| α [ml (g L⁻¹)⁻¹] | 4.5 | 36.6 |
| R²      | 0.9980 | 0.9975 |
| β [ml H₂ (g L⁻¹)⁻¹ h⁻¹] (linear regression) | 14 | 75.6 |
| R²      | 0.9942 | 0.9969 |

### Table 3. H₂-producing processes described by the original or modified forms of the Luedeking-Piret (LP) model.

| Biomass                | α value (ml g⁻¹ VSS⁻¹) | β value (ml H₂ g⁻¹ dw h⁻¹) | Reactor type | substrate | Fermentation/LP model | Ref. |
|-----------------------|------------------------|-----------------------------|--------------|-----------|-----------------------|------|
| *R. palustris* B10    | 6.85<sup>a</sup>       | 0.41<sup>b</sup>           | batch        | glycerol  | Light/modified        | [25] |
| *R. capsulatus*       |                        |                             |              |           |                       |      |
| B10                   | 4.5<sup>c</sup>        | 17<sup>d</sup>             | batch        | glucose   | Dark/original         | [30] |
| IR3                   | 36.6                   | 72<sup>d</sup>             |              |           |                       |      |
| Mangrove sediments    | 11.04                  | 0                           | batch        | glucose   | Dark/×<sub>rx</sub> = DoX | [36] |
| Local sewage sludge   | 224                    | 0                           | continuous   | sucrose   | Dark/×<sub>rx</sub> = DoX | [37] |
| Anaerobic sludge      | 759                    | 0                           | batch        | glucose   | Dark/original         | [38] |
| Anaerobic sludge      | 793                    | 0                           | batch        | glucose   | Dark/original         | [38] |
| Enterobacter cloacae  | 166<sup>e</sup>        | 0                           | batch        | xyllose   | Dark/DP/dt = α(1/X.dX/dt) | [40] |
| Clostridium pasteurianum | 918                  | 0                           | batch        | sucrose   |                       |      |
|                       | 876                    | 0                           |              |           |                       |      |

<sup>a</sup> Volatile Suspended Solids.
<sup>b</sup> undefined.
<sup>c</sup> α = 1/Y<sub>XS</sub> expressed in ml H₂ g protein L⁻¹ and β in ml H₂ (g L⁻¹)⁻¹ h⁻¹.
<sup>d</sup> ml H₂ g⁻¹ cell mass.
<sup>e</sup> D = liquid phase dilution rate, X = biomass concentration.
Table 4. H2-producing processes based on fed-batch regime. These processes have been previously summarized by Argun and Kargi [20], Androga et al. [41], Sagnak and Kargi [42], Basak et al. [16], and Uyar et al. [43].

| Strains | Carbon and nitrogen sources | Photobioreactor characteristics and operating conditions | Fed batch, semi continuous conditions | Maximal H2 productivity<sup>a</sup> (mM H2 L<sup>−1</sup> medium h<sup>−1</sup>) | Substrate conversion efficiency (mol H2 mol<sup>−1</sup> carbon source) | Ref. |
|---------|-----------------------------|------------------------------------------------------|--------------------------------------|---------------------------------|--------------------------------|------|
| R. sphaeroïdes B6 (thermostable) | Lactate (25 mM) Na Glutamate (5 mM) | 6-L plate polyacrylate outdoor | 15 % dilution/4 days | 1.07-1.56 (fine weather) 0.61-1 (cloudy weather) 0.09-0.56 (rainy weather) | 3.51<sup>b</sup> | [44] |
| Rhodopseudomonas capsulata | Glucose DFE<sup>e</sup>; acetate (8.5 mM) propionate (1.7 mM) butyrate (13.6 mM) Na Glutamate (3 mM) | 1.5-L indoor | HRT<sup>f</sup>, 72 h | 0.65 | 1.6 (acetate) 2.8 (propionate) 4 (butyrate) | [45] |
| Rhodopseudomonas palustris 42OL | Malic acid (24.3 mM) Glutamic acid (5.6 mM) | 1.07 L cylindrical glass pH 6.8 | ND small concentrated stock solution volume replacing sampling volume | 0.49 | ND | [46] |
| Rhodopseudomonas palustris sp. | Acetate (66.7 mM) Glutamic acid (5.6 mM) | Cylindrical glass (0.22 L) pH 6.8-7.2 4 W m<sup>−2</sup> indoor | 10% withdraw (experimental condition SC 240h) | 0.39 | ND | [17] |
| Clostridium acetobutylicum DSM792 + R. sphaeroïdes O.U.O O 1 | Non pretreated wheat starch from corn (C source)/yeast extract (0.5<sup>c</sup>) and peptone (1)<sup>c</sup> | Cylindrical reactor (0.25 L/0.12 <sup>d</sup>) controlled pH 7 192 W/m<sup>2</sup> Light/Dark ratio<sup>e</sup> = 2 | OLR<sup>b</sup> 1.5 g starch L<sup>−1</sup> d<sup>−1</sup> 2.5 % volume of medium replaced every day 0.575 g OLR L<sup>−1</sup> d<sup>−1</sup> | 1.03 | 2.62 (average on 33 days) | [47] |
| Clostridium butyricum N1VLB-B-3060 + R. sphaeroïdes VRM-3050 | Starch (4.5<sup>c</sup>)/yeast extract (0.04)<sup>c</sup>, Na Glutamate (0.9)<sup>c</sup> | Hungate tube (16 mL/8 mL)<sup>f</sup> Microaerobic conditions pH7.5 30 W/m<sup>2</sup> Light/Dark ratio<sup>e</sup> = 2.28 | 95-96 % volume of medium replaced | ND | 5.2 | [48] |

<sup>a</sup> calculated from data.  
<sup>b</sup> Dark Fermentation Effluent.  
<sup>c</sup> Hydraulic Retention Time.  
<sup>d</sup> Semi continuous, culture volume withdraw.  
<sup>e</sup> expressed in g L<sup>−1</sup>.  
<sup>f</sup> working volume/total volume of reactor.  
<sup>g</sup> Light fermentative biomass/Dark fermentative biomass concentration ratio.  
<sup>h</sup> Organic Loading Rates.

Product/biomass. Hydrogen yields can be expressed according to different form like g or L hydrogen per g<sup>−1</sup> biomass, or by lactate conversion rate. The latter was based on the theoretical total conversion (100%) of 1 mol of lactate leading to the formation of 6 mol of hydrogen. For both strains, the first step of culture corresponding to batch conditions led to a weak lactate conversion, 4.5 and 18.6% of lactate conversion, respectively. During the quasi-continuous process, B10 strain exhibited a weak lactate conversion rate compared to the over-producer strain IR3, 26.7% and 95%, respectively. These values were equivalent to 0.36 and 1.1 L H2 g<sup>−1</sup> lactate, representing 27 mg and 110 mg H2 g<sup>−1</sup> lactate, for B10 and IR3 strains, respectively.

It is essential to point out the assessments of Luedeking-Piret parameters in the literature in order to understand the values obtained in this study. Table 3 summarizes the H2 production processes, based either on dark or light fermentation, and modeled by the Luedeking-Piret model. The modeling of dark fermentation processes only implied the hydrogen production associated to the biomass growth; β value was equal to zero. At the opposite, the H2-producing process based on photosynthetic biomass involved both a non-growth and a growth-associated hydrogen production. Our results are the first study devoted to the robust estimation of λ, µ<sub>max</sub>, α and β parameter. It shows that β parameter is main parameter to describe kinetic of gas production by photofermentation, therefore dark anaerobic conditions present lower potential of volatile fatty acid conversion into hydrogen than light anaerobic conditions. In addition, the production rates of hydrogen by R. capsulatus with respect to light intensity irrespective of cell concentration have been previously described by Obeid et al. [28] and Androga et al. [34]. Authors showed that the specific hydrogen production rate (ml h<sup>−1</sup>) is proportional to the light intensity, which is the multiplying factor applied to non-growth-associated term (β) of Luedeking-Piret model.

Since the first study [44] devoted to the outdoor H2 production during a fed-batch culture of Rhodopseudomonas sphaeroïdes, many studies with PNS (purple-non-sulfur) bacteria have been carried out in different culture modes including fed-batch, repeated conditions or semi and continuous condition. They were summarized by Argun and Kargi [20], Androga et al. [41], Sagnak and Kargi [42], Basak et al. [10], and Uyar et al. [43]. Table 4 incorporates these and more recent data based on fed-batch and semi-continuous H2 production processes using either pure PNS cultures or co-cultures with dark fermentative bacteria. Indoor conditions represented the majority of previous fed-batch studies, using different light source and intensity as tungsten, halogen, fluorescent, incandescent, and Na vapor light. Nature and use of different unities of
illuminated increased the difficulty to compare experimental conditions. Recently many outdoor operating system for H2 production by R. capsulatus were carried out according to reactor design, effluent nature, bacterial strains, and feeding strategy conditions (Table 5), principally with Rhodobacter capsulatus.

Efficiency of H2 producing bioprocess based on PNS was habitually characterized by two principal parameters: the H2 productivity expressed as mM H2 produced L-1 h-1 and the substrate conversion ratio, which is the experimental H2 yield divided by theoretical yield. H2 productivity and substrate conversion ratio depend on experimental conditions as substrate nature, illumination, bacterial strain and feeding strategy conditions as indicated in previous summary tables extracted from recent reviews. In order to make the comparison more concise only results with Rhodobacter capsulatus strains were reported here. The production of H2 by PNS totally depends on the enzymatic activity of nitrogenase. However, H2 can be consumed by another enzymatic system known as uptake hydrogenase (hup). The productivity values for the wild type strain B10 and the over-producer strain IR3, extracted from Figure 3; were 0.26 and 0.41 mM H2 L-1 h-1, respectively. Substrate conversion ratio was calculated from oxidized lactate during semi-continuous conditions. The Luedeking-Piret model was used in this study to describe the bacterial growth, substrate consumption, and hydrogen gas production by using the purple non-sulfur bacterium Rhodobacter capsulatus. Hydrogen production by R. capsulatus during a quasi-continuous culture over a 150–200 h period was successfully controlled with various key parameters as inlet flow rate, feed volume, and dilution rate of substrate supply. The R. capsulatus over-producing strain IR3 displayed a greater hydrogen production yield correlated to the non-growth associated term (β). A good agreement was obtained between the experiments and simulations with high regression coefficient values (R² > 0.99). Fitting procedure allows to relevant parameters such as the lag time (λ), the maximum growth rate (μmax), and both non-growth (β) and growth-associated terms (α) of Luedeking-Piret model. This parameter access will be useful for comparison between strains or/and literature data and for the automation and control of bioprocesses for the continuous fermentation process.

4. Conclusions

The Luedeking-Piret model was used in this study to describe the bacterial growth, substrate consumption, and hydrogen gas production by using the purple non-sulfur bacterium Rhodobacter capsulatus. Hydrogen production by R. capsulatus during a quasi-continuous culture over a 150–200 h period was successfully controlled with various key parameters as inlet flow rate, feed volume, and dilution rate of substrate supply. The R. capsulatus over-producing strain IR3 displayed a greater hydrogen production yield correlated to the non-growth associated term (β). A good agreement was obtained between the experiments and simulations with high regression coefficient values (R² > 0.99). Fitting procedure allows to relevant parameters such as the lag time (λ), the maximum growth rate (μmax), and both non-growth (β) and growth-associated terms (α) of Luedeking-Piret model. This parameter access will be useful for comparison between strains or/and literature data and for the automation and control of bioprocesses for the continuous fermentation process.

Declarations

Author contribution statement

Jonathan Deseure, Jean-Pierre Magnin: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Jamila Obeid: Conceived and designed the experiments; Performed the experiments.
John C. Willison: Analyzed and interpreted the data; Wrote the paper.
