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Multi-Objective Optimization of Thermophilic Biohydrogen Production

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Dynamic optimization of thermophilic hydrogen and by-product production requires the use of robust models coupled with control strategies. In this work, a model was used to optimize biohydrogen (bioH₂) and acetate production using *Thermotoga maritima* (*T. maritima*) with a Multi-Objective Optimization (MOO). The aim was to find the trade-off between the maxima of yield and productivity of the hydrogen production and acetate in a continuous dark fermentation process by modifying the inlet liquid flow rate. A dynamic mass balance model was used to optimize thermophilic bioH₂ and acetate production using *T. maritima* MSB8 (DSMZ 3109) strains. In this study, the simulations were run using an Intel® Core™ i7-9750 H @ 2.60 GHz, 16 GB RAM computer. The *paretosearch* integrated function from MATLAB® was used to obtain Pareto Optimal Sets (POSS) for each inlet substrate concentration (S_{in}). Some constraints were used to fit to the model dynamics of dark fermentation performed by *T. maritima*. using glucose as a substrate; the maximum theoretical hydrogen yield was 4 mol H₂/mol glucose at 80 °C. The MOO was performed for five different substrate concentrations. The maximum Euclidean distance (d_{max}) from the origin of the normalized coordinates was used to select the Pareto Optimal Points (POPs). The simulation results indicate that an S_{in} equal to 70×10^{-3} mol/L is optimum to significantly maximize/minimize Y at 3.48 and 0.33 mol/mol_{glucose_{in}} and P at 3.49×10^{-3} and 1.66×10^{-3} mol/L/L_{reactor} · h simultaneously, respectively, for hydrogen and acetate production.

1. Introduction

Dynamic models are commonly used to understand and predict the outcomes of biological processes in various industries, such as wastewater treatment, food production, and bioenergy production (Kim et al., 2018). One of the most important biological processes in bioenergy production is the conversion of organic matter into biogas using microorganisms. Dark fermentation of microorganisms leading to bioH₂ production is an example that has been actively investigated for many years for its reported high energy yields (Chandrasekhar et al., 2015). However, many challenges are hindering the full control of the process for scale-up and industrialization perspectives due to the complex nature of the system and the presence of multiple variables that heavily impact the outcomes. A variety of ways have been identified to improve the bioH₂ production of microorganisms, including process optimization through predictive and dynamic models. Such models can help further overcome the challenges faced by the bioH₂ production industry by informing control strategies that can optimize multiple variables simultaneously. A popular optimization strategy is Multi-Objective Optimization (MOO), which involves optimizing multiple variables that are usually conflicting, such as yield and productivity. MOO is commonly used in the industry to optimize processes where there are multiple objectives, such as reducing energy consumption, minimizing waste, and maximizing profitability (Chang et al., 2015; Vertovec et al., 2021).

MOO generates a Pareto Optimal Set (POS), which is a set of optimal solutions, Pareto Optimal Points (POP), for different objectives that cannot be improved without sacrificing the performance of other objectives.

This simulation outputs a Pareto front that predicts chosen parameters of a bioprocess (Islam et al., 2021). In this work, a mathematical model describing dark fermentation was combined with MOO dynamics. A published model of a hyperthermophile strain has been used and a previously reported MOO methodology was followed (Auria et al., 2016; Acosta-Pavas et al., 2022). Auria et al. (2016) model has been validated experimentally on a variety of substrates and has proven its capability to simulate and predict the conversion of organic matter into bioH₂ with high accuracy.

This study proposes a MOO approach to find the trade-off between the maximum yield and productivity of the biogas and a by-product production process, using a POS. Next, following the desired criteria, POPs are selected from the set and used as a reference trajectory for experimental applications and a tool for improving day-to-day performance management at a laboratory or industrial scale.

2. Biohydrogen Model Extension Proposal

Dark fermentation by biomass conversion is considered the most promising method, and the most understood process for bioH₂ production (Froio and Bezerra, 2021). Dark fermentation is a process in which complex organic substrates are converted in the absence of light, by anaerobic heterotrophic microorganisms, to bioH₂, CO₂, and various Volatile Fatty Acids (VFAs) (Eq(1) and Eq(2)) (Patel et al., 2018). The theoretical maximum yield of 4 mol H₂/mol hexose (Thauer et al. 1977) can be achieved when 2 moles of acetate is produced during dark fermentation (see Eq(1)).



However, this maximum yield (or Thauer limit) can only be achieved with thermophilic H₂-producing microorganisms. Most of these thermophilic microorganisms can hydrolyze various polysaccharides and ferment the released hexoses and pentoses. So far, the hydrogen-producing (hyper)thermophilic microbial species studied belong essentially to the classes Clostridia, Thermotoga, and Thermococcales (Pradhan et al., 2015). Thermotoga, hyperthermophile marine bacteria, are considered the preferred option for bioH₂ production and industrial purposes since they can produce high H₂ yields (2.4 to 3.85 mol H₂/mol hexose) from many organic wastes (Saidi et al., 2018). Overall, H₂ production yields from dark fermentation by pure cultures such as *T. maritima* has been reported to reach up to 3.05 mol H₂/mol hexose. *T. maritima* is a hyperthermophile bacterium found in marine environments and is known to produce hydrogen yields close to the thermodynamic limit (Thauer limit) from a large variety of substrates (Boileau et al., 2016). However, acetate, but also lactate, are the main organic products of the fermentation performed by *T. maritima*. Indeed, when the culture conditions are not optimal, *T. maritima* redirects the reoxidation of its cofactors towards the production of lactate, thus limiting the production of bioH₂. *T. maritima* has been extensively studied and a modeling approach to various batch experiments was carried out by Auria et al. (2016) (Auria et al., 2016). The batch experiments were carried out in a 2 L bioreactor with a working volume (*V*_{liq}) of 1.5 L and lasted for 10.1 h operating at 80 °C. The initial substrate concentrations were: 14 × 10⁻³ mol/L of glucose, 1 g/L of yeast extract, and 0.12 g/L of thiosulfate. These values are reported in Table 1. The inlet gas flow rate and stirring were maintained constant throughout the entire experiment at 8.33 m³/s and 36.65 rad/s, respectively.

Table 1: Experimental data from the literature (Auria et al., 2016)

Biomass		Substrate concentration × 10 ⁻³ (mol/L) ; (g/L)			Product concentration in the liquid phase × 10 ⁻³ (mol/L)		Product concentration in the gas phase × 10 ⁻³ (mol/L)	
Time (h)	X (g/L)	Glucose	Yeast Extract	Thiosulfate	Acetate	Lactate	H ₂	CO ₂
0	0.053	14	1	0.12	-	-	-	-
10.1	0.155	0	22	-	22	9	35	79

Following a previously published methodology, an extension of the Auria et al. (2016) model is proposed to convert the operational reactor mode from a batch to a continuous culture. Adjustments of the dynamic model were carried out and the inlet liquid flow rate with respect to the component concentration change was added to the equations. This was a necessary step to be able to apply a MOO strategy.

To present and simplify the changes made, we have defined the gas ($S_{gas,i}$) liquid ($S_{liq,j}$) and biomass growth (X) phases as state variables represented in Eq(1), Eq(2), and Eq(3), respectively as,

$$\frac{dS_{gas,i}}{dt} = N_i \left(\frac{V_{liq}}{V_{gas}} \right) - \frac{q_{gas}}{V_{gas}} S_{gas,i} \quad (3)$$

$$\frac{dS_{liq,j}}{dt} = \frac{q_{liq,in}}{V_{liq}} (S_{j,in}^l - S_{liq,j}) + \sum \beta_j \mu X - N_i \quad (4)$$

$$\frac{dX}{dt} = - \frac{q_{liq,in}}{V_{liq}} X + (\mu - \mu_d) X \quad (5)$$

where the sub-indexed variables are: $i = H_2, CO_2$, and H_2S in the gas phase; $j =$ glucose, yeast extract, thiosulfate, acetate, lactate, extracellular polysaccharides, H_2, CO_2 , and H_2S in the liquid phase. V_{gas} and V_{liq} = volume of the gas and liquid fraction, respectively; β_j = the stoichiometric coefficient of components j ; $j = S_{gas,i}$ and $S_{j,in}^l$ = outlet and inlet concentrations of component i and j in the gas and liquid phases, respectively; X = is the concentration of biomass; N_i = the mass transfer of component i ; q_{gas} : and $q_{liq,in}$ = the total outlet gas and inlet liquid flowrates, respectively; μ and μ_d = the specific growth and death rates constants of biomass.

3. Multi-Objective Optimization

Multi-Objective Optimization (MOO) implies solving multiple problems simultaneously where more than one variable is to be optimized (Chang, 2015; Vertovec, Ober-Blöbaum Margellos, 2021). Usually, variables to be optimized in a bioprocess are conflicting. Productivities, yields, experimental stage, and process durations are examples of variables that are usually conflicting and therefore difficult to optimize. Ultimately, the goal of MOO dynamics is to find a balance that maximizes the targeted outcomes of the process. The outcome of a MOO model simulation is a Pareto front. A Pareto front allows the visualization of the obtained Pareto Optimal Set (POS). A POS is an optimal solution set composed of multiple Pareto Optimal Points (POPs). Lastly, POPs are parameter values, indicators, or coordinates that fulfill the desired conditions of conflicting variables (Deb et al., 2002). Generally, a MOO can be formulated as,

$$\min_{x,u,p,t} \{Y_1(x,u,p), \dots, Y_m(x,u,p)\} \quad \text{subject to} \quad \begin{cases} dx/dt = f(x,u,p,t) & t \in [0, t_f] \\ f_i(x,u,p,t) \leq 0 & i = 1, 2, \dots, n \\ g_i(x,u,p,t) = 0 & i = 1, 2, \dots, k \\ u^L \leq u \leq u^U \end{cases} \quad (6)$$

where $Y_1, \dots, Y_m = m$ objective functions; x = to the state variables; u and p = the control variables and parameters; f_i and g_i = inequality and equality constraints on the variable states; u^L and u^U = indicate the control variables' lower and upper bounds (Tsiantis et al., 2018).

Based on the desired outcomes, a MOO strategy is proposed to identify the best possible values for the objective functions. The MOO methodology comprises three main steps: firstly, defining the model that represents the biological process, secondly, specifying the objective functions $Y_1, \dots, Y_m$ to be minimized or maximized, including the control variables u and the constraints that are lower and upper bounds u^L and u^U , selecting the conflicting variables to be optimized for the objective functions $Y_1^*, \dots, Y_m^*$ which will be used as a reference in the optimization process. Finally, a POS is obtained from which one or more POPs are selected following certain criteria defined by the end user.

4. Case Study: Multi-Objective Optimization in Dark Fermentation Bioprocess

The primary goal of the study was to optimize, independently, H_2 and acetate production processes by maximizing their respective yields (Y_{H_2} and $Y_{Acetate}$) and productivities (P_{H_2} and $P_{Acetate}$). To reach this goal, it proposes different sets denoted Pareto optimal sets (POS) of POPs following two chosen control variables: the inlet liquid concentration S_{in} and liquid flow rate $q_{liq,in}$. This would allow the user to minimize experimental costs by developing an experimental design based on the experimental conditions forecasted by the model. To optimize hydrogen production, Y_{H_2} was defined as the amount of hydrogen production rate (q_{gas,H_2}) over S_{in} , while P_{H_2} represents the ratio of q_{gas,H_2} to V_{liq} , as follows.

$$Y_{H_2} = \frac{q_{gas,H_2}}{S_{in}} \quad (7)$$

$$P_{H_2} = \frac{q_{gas,H_2}}{V_{liq}} \quad (8)$$

As for acetate, Y_{acet} was defined as the final concentration of acetate produced ($S_{Acet,out}$) over S_{in} , while P_{Acet} represents the ratio between $S_{Acet,out}$ multiplied by $q_{liq,in}$ and V_{liq} expressed as,

$$Y_{Acet} = \frac{S_{Acet,out}}{S_{in}} \quad (9)$$

$$P_{Acet} = \frac{S_{Acet,out} \times q_{liq,in}}{V_{liq}} \quad (10)$$

where (q_{gas,H_2}) was calculated as,

$$q_{gas,H_2} = q_{gas} \left(\frac{P_{gas,H_2}}{P_{gas}} \right) \quad (11)$$

with P_{gas} and P_{gas,H_2} = the total pressure in the bioreactor and partial pressure of H_2 in Pa .

In this work, multiple simulations were run using an Intel® Core™ i7-9750H @ 2.60 GHz, 16 GB RAM computer. The *paretosearch* function provided by MATLAB® was used to obtain Pareto optimal set for each S_{in} . The MOO was proposed as,

$$\max_{\{q_{liq,in}\}} (Y_{H_2}, P_{H_2}) \quad \text{subject to} \begin{cases} \text{Equations (3) – (8) and (11)} \\ Y_{H_2} \leq 4 \text{ [mol } H_2/\text{mol glucose]} \\ 1 \times 10^{-3} \leq q_{liq,in} \leq 1 \text{ [L/h]} \end{cases} \quad (12)$$

$$\max_{\{q_{liq,in}\}} (Y_{Acet}, P_{Acet}) \quad \text{subject to} \begin{cases} \text{Equations (3) – (6) and (9) – (11)} \\ Y_{H_2} \leq 4 \text{ [mol } H_2/\text{mol glucose]} \\ 1 \times 10^{-3} \leq q_{liq,in} \leq 1 \text{ [L/h]} \end{cases} \quad (13)$$

where Eq(12) and Eq(13) state the constraints that were used where Eq(3) – Eq(5) represent the model dynamics and 4 $\text{mol } H_2/\text{mol glucose}$ is the theoretical yield limit (Thauer limit) mentioned previously. The MOO was performed for five S_{in} concentrations such that $S_{in} [\times 10^{-3} \text{ mol/L}] \in \{14, 42, 70, 90, 110\}$. The POS was composed of 60 points for every S_{in} .

5. Selection of Pareto Optimal Points (POPs)

A normalization of POSs [0, 1] was performed in order to perform the selection. Three POPs were chosen to maximize the yield and productivity: the first POP to maximize productivity, the second to maximize Euclidean distance (d_{max}) from the origin to the standardized coordinates, and the third to maximize yield. The results are summarized in Table 2.

Table 2. Multi-Objective optimization results.

Substrate concentration (S_{in}) ($\times 10^{-3} \text{ mol/L}$)	POP			
	for maximum Yield (Y)			
	for hydrogen		for acetate	
	Y_{H_2} ($\text{mol}_{H_2}/\text{mol}_{Glucose_{in}}$)	$P_{H_2} \times 10^{-3}$ ($\text{mol/L}_{H_2}/L_{reactor} \cdot h$)	Y_{Acet} ($\text{mol}_{Acet}/\text{mol}_{Glucose_{in}}$)	$P_{Acet} \times 10^{-3}$ ($\text{mol/L}_{Acet}/L_{reactor} \cdot h$)
14	3.9906	2.3542	1.4678	0.2141
42	3.8406	3.3605	1.2171	0.2536
70	3.9550	3.4624	0.6380	0.5583
90	3.9302	3.4927	0.4329	0.6313
110	3.9644	3.5074	0.3473	0.6511
	for maximum Euclidean distance (d_{max})			
14	3.2867	2.4859	1.1690	0.9550
42	3.5509	3.3987	0.7952	1.3254
70	3.5566	3.5125	0.5209	1.3327
90	3.7967	3.4993	0.2684	1.5657
110	3.7917	3.5125	0.2574	1.3517

Substrate Concentration (S_{in}) ($\times 10^{-3} \text{ mol/L}$)	POP			
	for maximum productivity(P)			
	for hydrogen		for acetate	
	Y_{H_2} ($\text{mol}_{H_2}/\text{mol}_{Glucose_{in}}$)	$P_{H_2} \times 10^{-3}$ ($\text{mol}/L_{H_2}/L_{reactor} \cdot h$)	Y_{Acet} ($\text{mol}_{Acet}/\text{mol}_{Glucose_{in}}$)	$P_{Acet} \times 10^{-3}$ ($\text{mol}/L_{Acet}/L_{reactor} \cdot h$)
14	2.8644	2.5085	0.7319	1.2542
42	2.9857	3.4261	0.5817	1.4420
70	3.4779	3.4857	0.3068	1.6610
90	3.6579	3.5023	0.1872	1.6956
110	3.7199	3.5130	0.1418	1.6949

Figures 1-(A) and (B) show the POSs of hydrogen (left) and acetate (right) for the different initial substrate concentrations tested. The selected POPs were highlighted in orange, yellow, and purple shapes for maximum Y , d_{max} and P , respectively. The result of the simulation shows that POSs ranges are narrower as we increase S_{in} and Y_{H_2} , P_{H_2} , and P_{Acet} increases in contrast to Y_{Acet} . However, the rise for P_{H_2} and P_{Acet} ranges are noticeable from $S_{in} \geq 42 \times 10^{-3} \text{ mol/L}$ and was almost unchanged beyond $70 \times 10^{-3} \text{ mol/L}$. The same behavior is observed for Y_{H_2} and Y_{Acet} POS boundaries from $S_{in} \geq 70 \times 10^{-3} \text{ mol/L}$. The simulation results indicate that an S_{in} equal to $70 \times 10^{-3} \text{ mol/L}$ is optimum to significantly maximize/minimize Y and P simultaneously for both products. Moreover, studies of continuous-flow dark fermentation reported that at higher carbohydrate (organic) loading rates, bioH₂ production is inhibited by the overaccumulation of hydrogen gas in the working volume (Van Ginkel and Logan, 2005). It is important to mention that this parameter is taken into account in our proposed model and during our simulations and therefore could explain our results. Thus, the increase of S_{in} beyond $70 \times 10^{-3} \text{ mol/L}$ indicates that the carbohydrate source, glucose, is not the limiting factor in those conditions and bioH₂ production could solely be limited by *T. maritima* growth rate.

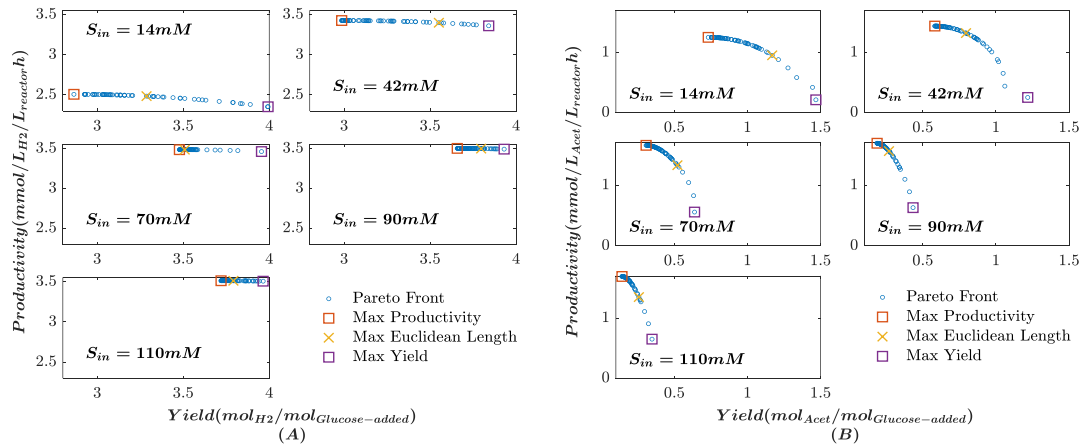


Figure 1: (A). POSs for $S_{in} [\times 10^{-3} \text{ mol/L}] \in \{14, 42, 70, 90, 110\}$, and selected POPs for yield and productivity maximization and maximum Euclidean length for hydrogen and (B). acetate produced.

6. Conclusion

A MOO strategy for the bioH₂ production process based on the mathematical model used for the two objectives (maximization of Y and P) was successfully developed for both hydrogen and acetate production.

We analyzed 10 cases, where it was observed that maximizing P lowers Y ratio and vice versa. It is observed that by increasing the substrate concentration (S_{in}) of the inlet liquid, maximum productivity increases whereas maximum yield decreases for acetate. It was also shown that the hydrogen yield is improved by increasing the substrate concentration (S_{in}) of the inlet liquid. The simulation results indicate that an S_{in} equal to $70 \times 10^{-3} \text{ mol/L}$ is optimum to significantly maximize/minimize Y and P simultaneously for both products. Future work includes the validation of the model using experimental data, the application of the dynamic MOO methodology, and the use of an economic Pareto, allowing the estimations of the purchase price of substrates or the sale of bioH₂ or acetate.

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