Review

Hydrogen Production from Biomass and Organic Waste Using Dark Fermentation: An Analysis of Literature Data on the Effect of Operating Parameters on Process Performance

Rita Noelle Moussa [1], Najah Moussa [2] and Davide Dionisi [1,*]

1. Chemical Processes and Materials Group, School of Engineering, University of Aberdeen, Aberdeen AB24 3UE, UK; r.moussa.19@abdn.ac.uk
2. Department of Statistics, The Higher Institute of Applied and Economic Sciences (ISSAE-CNAM), Beirut 1103 2100, Lebanon; najahmoussa8@gmail.com
* Correspondence: davidedionisi@abdn.ac.uk; Tel.: +44-(0)1224-272814

Abstract: In the context of hydrogen production from biomass or organic waste with dark fermentation, this study analysed 55 studies (339 experiments) in the literature looking for the effect of operating parameters on the process performance of dark fermentation. The effect of substrate concentration, pH, temperature, and residence time on hydrogen yield, productivity, and content in the biogas was analysed. In addition, a linear regression model was developed to also account for the effect of nature and pretreatment of the substrate, inhibition of methanogenesis, and continuous or batch operating mode. The analysis showed that the hydrogen yield was mainly affected by pH and residence time, with the highest yields obtained for low pH and short residence time. High hydrogen productivity was favoured by high feed concentration, short residence time, and low pH. More modest was the effect on the hydrogen content. The mean values of hydrogen yield, productivity, and content were, respectively, 6.49% COD COD^{-1}, 135 mg L^{-1} d^{-1}, 51% v/v, while 10% of the considered experiments obtained yield, productivity, and content of or higher than 15.55% COD COD^{-1}, 305.16 mg L^{-1} d^{-1}, 64% v/v. Overall, this study provides insight into how to select the optimum operating conditions to obtain the desired hydrogen production.

Keywords: hydrogen; dark fermentation; organic waste; statistical analysis; regression model

1. Introduction

Anaerobic digestion (AD) is an established process used across the world for the conversion of biomass and organic waste into methane, a renewable energy vector [1–3]. While the use of AD to produce methane is an established commercial process, to the best of our knowledge, there are, to date, no full-scale processes in which AD, also called dark fermentation (DF) in this case, is used for the primary purpose to produce the intermediates of the process, i.e., hydrogen and short-chain organic acids (SCOAs). This is despite the large volume of recent research, at lab or pilot scale, on DF to produce these intermediates [4].

Anaerobic digestion consists of several steps. Hydrolysis of polymeric biomass components, e.g., proteins, to their monomers is the first step and is often rate limiting [4]. During the second step, also called acidogenesis, various compounds (hydrogen, SCOAs, alcohols) are produced as intermediates. Generally, in conventional AD, these compounds are converted to methane in the final step of methanogenesis. However, the intermediates produced in the second step can be more economically valuable than the end product methane [5].

This study focuses on the use of AD or DF to produce hydrogen. Hydrogen gas is considered a replacement for fossil fuels in the future because of its environmental and socio-economic advantages [6]. To date, hydrogen has been produced at a commercial scale at global rates, in the order of 50 million tons per year, and is mainly used in chemical

synthesis, e.g., for ammonia and methanol synthesis and hydrogenation reactions. There is increasing interest in the use of hydrogen as a renewable energy vector when produced from water electrolysis using renewable electricity. However, currently, most of the hydrogen produced comes from natural gas, which is a non-renewable, therefore non-sustainable, fossil resource, at least in the medium-to-long term [7].

The production of hydrogen from DF of biomass or organic waste has several environmental advantages, compared with its production from natural gas or from water electrolysis. Compared with the use of natural gas, DF uses renewable resources or, even better, wastes that need to be treated. To produce hydrogen, the DF of organic waste can represent the first stage of the waste treatment and valorisation process. In addition, and in contrast with the chemical synthesis of hydrogen from natural gas (steam methane reforming), DF uses mild temperatures close to ambient values and does not need the external addition of metal catalysts, with clear environmental benefits. Compared with the use of water electrolysis, DF is less energy intensive. Considering several renewable energy scenarios for the UK, our recent study has shown that energy from organic waste, including hydrogen production from DF, could potentially provide up to ~30% of the heating and transport energy in this country [8].

However, despite the potential environmental benefits, hydrogen production from DF of biomass and organic waste is still limited at the lab or pilot scale [9]. In order to have a successful commercial process, it is necessary to maximise the hydrogen yield, productivity, and content in the fermentation gas. High yield is important to maximise the hydrogen produced from a given mass of biomass/waste. High volumetric productivity corresponds to a high hydrogen production rate per unit volume of the reactor and is therefore essential to minimise the volume of the equipment and the capital costs of the process. Hydrogen content in the fermentation gas should also be maximised to minimise the separation costs due to the removal of other components, usually mainly carbon dioxide.

Many process parameters can affect hydrogen yield, productivity, and content. The feedstock concentration is expected to affect hydrogen productivity while potentially also affecting the yield because of inhibition effects. The residence time can affect the extent of biomass degradation and the possible conversion of hydrogen into methane by methanogenic microorganisms. The residence time also can directly affect the process productivity. As with any other biological process, DF can be affected by pH and temperature.

This study aims to analyse literature data on hydrogen production from DF, investigating the effect of the main process parameters, i.e., feedstock concentration, residence time, pH, and temperature on hydrogen yield, productivity, and content in the fermentation gas. The study aims to develop a quantitative analysis of the data, also using a statistical multi-linear regression model. Overall, this study contributes to understanding which parameters are more important in determining the performance of DF and provides insight into how to choose the process parameters to obtain the desired performance.

2. Materials and Methods

2.1. Data Prospection

Articles were researched using the words 'organic waste', 'dark fermentation', and 'hydrogen gas', then filtering all those which could provide enough information on the process conditions and data results, to be able to evaluate the parameters of interest. The list of all the studies considered, with the operational parameters investigated, and analysed process parameters, is reported in Table S1 of the Supplementary Materials [10–64].

After data collection, 55 articles (339 experiments) were used to analyse the effect on hydrogen yield (% COD COD^{-1}), hydrogen productivity (mg L^{-1} d^{-1}), and hydrogen content (% v/v) of various experimental parameters—namely, operation mode, substrate composition, substrate concentration or total chemical oxygen demand (tCOD gCOD L^{-1}), experimental length for batch mode experiments, and hydraulic retention time for a continuous mode, which were merged into one parameter named residence time (d), temperature (°C), pH, inoculum pretreatment or methanogens inhibition, and substrate pretreatment.

Hydrogen yield was defined as the hydrogen produced per unit of substrate fed (% COD COD^{-1}). Hydrogen productivity was defined as the hydrogen produced per unit reactor volume per unit time (mg L^{-1} d^{-1}), and hydrogen content was defined as the hydrogen content in the biogas (% *v/v*).

In the calculation of the yield in COD units, hydrogen was converted using the factor 8 g COD g H_2^{-1}, derived from the stoichiometry of hydrogen oxidation. When the COD of the feedstock was not reported in the papers, it was estimated from the composition of the feedstocks (Table 1).

Table 1. Conversion factors used in this study.

Conversion Unit	Conversion Factor	Reference
gCOD gVS^{-1} carbohydrates	1.13	[65]
gCOD gVS^{-1} proteins	1.6	[65]
gCOD gVS^{-1} lipids	2.03	[65,66]
gCOD gDS^{-1} wastewaters	1.2	[67]

2.2. Regression Modelling and Statistical Analysis

The normality of the hydrogen yields, hydrogen productivity, and content was carried out using the Anderson–Darling test. The test is appropriate to test normality for relatively large samples sizes (*n*) (i.e., larger than 10). The Anderson–Darling test rejects the hypothesis of normality when the *p*-value is less than or equal to 0.05.

Non-parametric tests (the Kendall rank test for continuous variable and the Kruskal–Wallis rank test for categorical variables) were performed to check if there is a significant relationship between the dependent (hydrogen yield, productivity, and content) and independent variables (temperature, pH, residence time, substrate concentration, substrate type, substrate pretreatment, and methanogens' inhibition). The data coding of each independent variable are listed as follow:

1. Operating mode: continuous (CN), batch (BA);
2. Residence time (RT): RT1 ≤ 2 d, 2 d < RT2 ≤ 10 d, RT3 > 10 d;
3. Substrate concentration as total chemical oxygen demand (tCOD);
4. Substrate type: soluble substrates (SS) made up of single substrates or synthetic models of wastewaters, non-soluble substrates (SNS) made up of biomass, sludge, or organic/food waste;
5. Pretreatment of the substrate: yes (SY), no (SN);
6. Methanogens' inhibition: yes (IY), no (IN);
7. Temperature: mesophilic (Tm), thermophilic (Tt);
8. pH: acidic (pHa < 6), neutral (6 ≤ pHn ≤ 8), alkaline (pHb > 8).

Total chemical oxygen demand is the only continuous independent variable, and the other 7 independent variables are categorial. These categorical variables take values of 0 or 1.

The relationship between the continuous variable tCOD with hydrogen yield, content, and productivity was tested with Kendall's tau test, to understand the strength of the relationship between the two variables. Kendall's tau-b (τb) correlation coefficient is a non-parametric measure of the association that exists between two variables and identifies its direction, either negative or positive, on at least an ordinal scale. Kendall's tau-b values are divided into the following four categories:

- τb < ±0.10, very weak;
- ±0.10 < τb < 0.19, weak;
- ±0.20 < τb < 0.29, moderate;
- ≥ −0.30, strong.

The relationship between the categorical variables (pH, temperature, residence time, substrate type, substrate pretreatment, and methanogens' inhibition) with hydrogen yield, productivity, and content was tested with Kruskal–Wallis one-way analysis of variance test

by ranks. The test compares two or more independent variables from identical or different sample sizes and identifies whether the samples are originally from the same or from a different distribution. Kendall's tau-b and Kruskal–Wallis constitute one of the bivariate analysis forms of quantitative analysis (or non-parametric analysis) (Table S2).

Afterwards, modelling was performed to correlate the effect of each operating parameter (independent variables) with hydrogen yield (% COD COD^{-1}), hydrogen productivity (mg L^{-1} d^{-1}), and hydrogen content (% v/v) as a response (dependent variables). The general linear model was performed using RStudio software 4.1.2 and calculated according to the 'ellipse' model (Table S3). The initial full regression model was developed according to Equation (1).

$$Y = \text{Constant} + A1 \times \text{pHa} + A2 \times \text{pHn} + A3 \times \text{pHb} + B1 \times \text{Tm} + B2 \times \text{Tt} + C \times \text{tCOD} + D1 \times \text{SS} + D2 \times \text{SNS} + E1 \times \text{SN} + E2 \times \text{SY} + F1 \times \text{IN} + F2 \times \text{IY} + G1 \times \text{BA} + G2 \times \text{CN} + I1 \times \text{RT1} + I2 \times \text{RT2} + I3 \times \text{RT3} \tag{1}$$

where A1, A2, A3, B1, B2, C, D1, D2, E1, E2, F1, F2, G1, G2, I1, I2, and I3 represent the model coefficients, which take the same unit as the dependent variable yield, productivity, and content.

To choose the best fit model, a backward elimination approach was used, starting with all independent variables, removing each variable one by one each time. The most statistically insignificant variables were removed first, and their loss did not affect the model fit. The process was repeated until no further variables can be removed without significantly and statistically altering the model fit. The significance of the variables was determined depending on the p-value. This later was divided into the following five categories:

-    Highly significant '***': $0 \leq p\text{-value} < 0.001$;
-    Fairly significant '**': $0.001 \leq p\text{-value} < 0.01$;
-    Significant '*': $0.01 \leq p\text{-value} < 0.05$;
-    Non-significant '•': $0.05 \leq p\text{-value} < 0.1$;
-    Highly non-significant 'no symbol': $p\text{-value} \geq 0.1$.

3. Results and discussion

3.1. General Observation and Descriptive Statistics

Table 2 shows various types of substrates used in the considered studies in the literature. The most frequent type of substrates was pure substrates, followed by biomass, food waste, and organic waste.

Table 2. Frequency and percentage of substrate types, grouped by category.

Substrate Category	Frequency	Percentage
Pure substrate (glucose, sucrose, starch)	96	28.2
Biomass (oat straw, corn, sugarcane, wheat, soybean, rice, cellulose)	79	23.2
Food waste (bean curd manufacturing, molasses, vinegar, mixed food waste)	47	13.8
Organic waste (waste ground wheat, waste potato starch, rice slurry, sugarcane bagasse, and poplar leaves	42	12.3
Carbohydrate-rich wastewater (sugar factory, condensed molasses, and sucrose-rich wastewater)	22	6.5
Industrial wastewater (rice winery, coffee drink manufacturing, brewery, citric acid, and distillery wastewaters)	21	6.2
Synthetic wastewater	13	3.8
Wastewater sludge	10	2.9
Municipal wastewater	10	2.9

3.2. Profiles of the Performance Variables vs. Operating Parameters

The relationship between the process performance variables (hydrogen yield, productivity, and content) and the operating parameters (tCOD, pH, temperature, and residence time) is shown in Figures 1–3. Parts (a,c,e,g) of Figures 1–3 show all the literature data, while parts (b,d,f,h) show average values for each narrow range of the operating parameters.
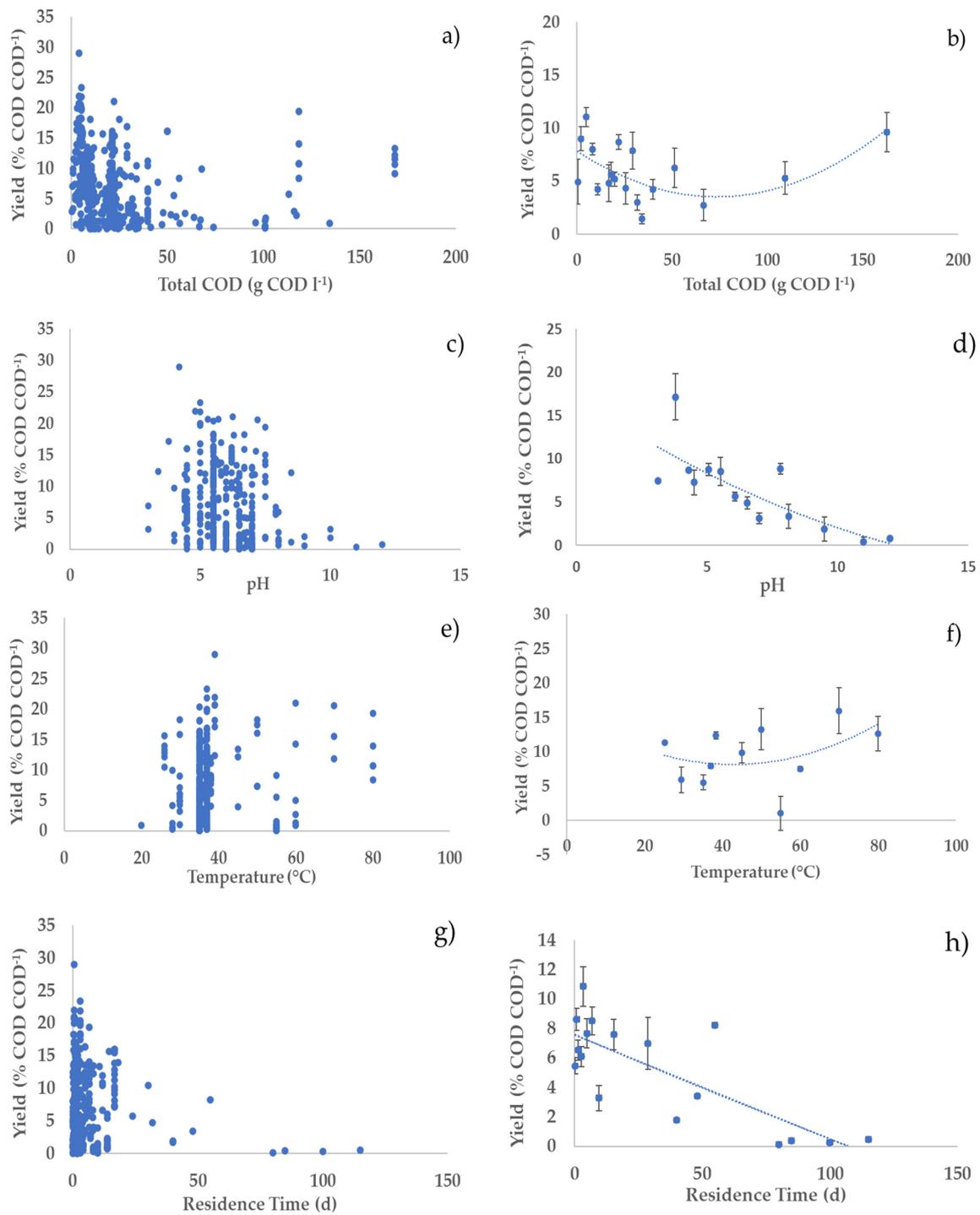
Figure 1. Relationships between hydrogen yield and operational parameters: (**a,c,e,g**) all considered experiments; (**b,d,f,h**) average values of experiments in each narrow range (width maximum 20%) of the operating parameters (error bars indicate the standard error) with second-order polynomial curve. The polynomial curve is only used to visualise the general trend, without any statistical significance.

Figure 2. Relationships between hydrogen productivity and operational parameters: (**a,c,e,g**) all considered experiments; (**b,d,f,h**) average values of experiments in each narrow range (width maximum 20%) of the operating parameters (error bars indicate the standard error) with second-order polynomial curve. The polynomial curve is only used to visualise the general trend, without any statistical significance.
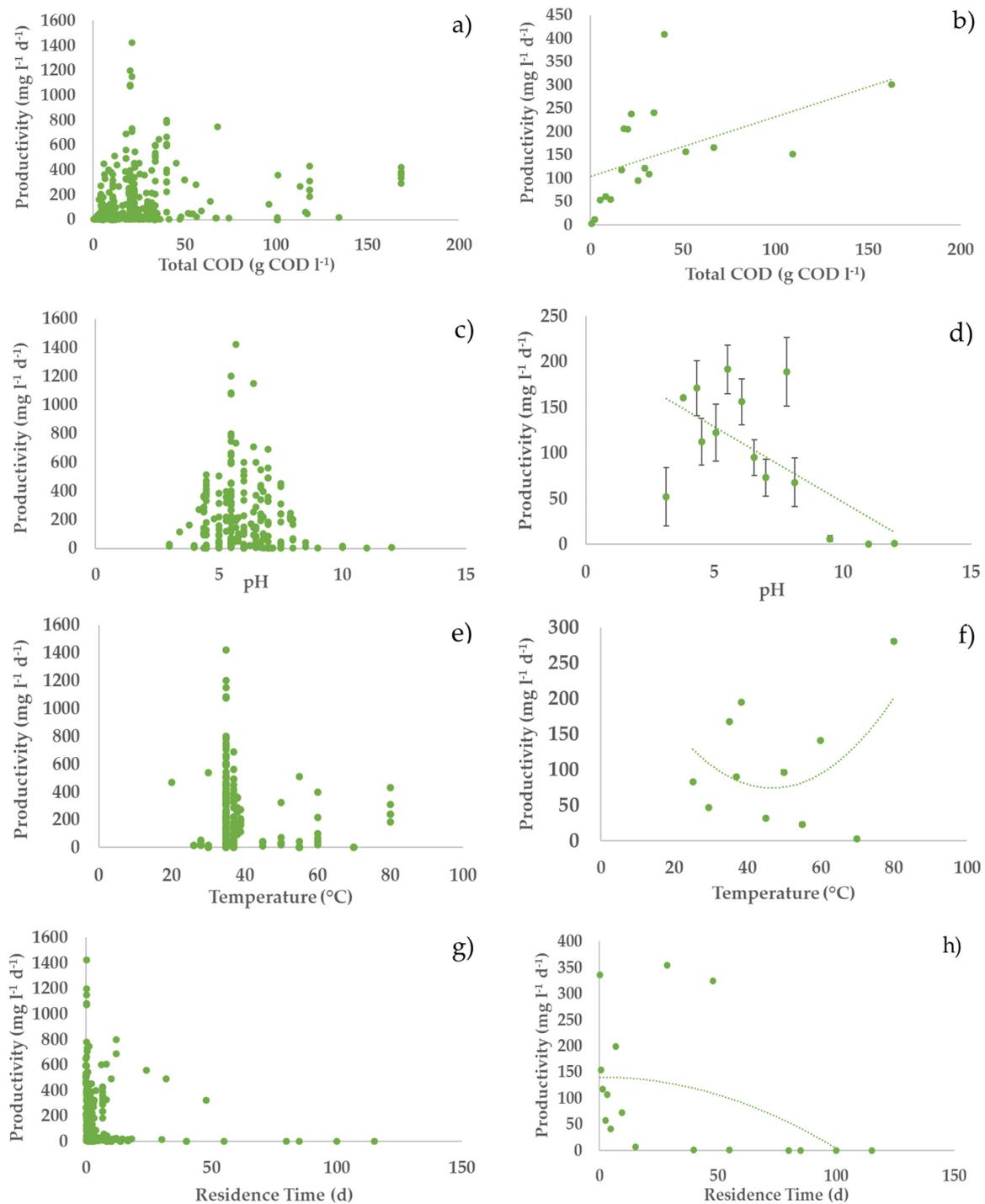
Figure 3. Relationships between hydrogen content in biogas and operating parameters: (**a**,**c**,**e**,**g**) all the considered experiments; (**b**,**d**,**f**,**h**) average values of experiments in each narrow range (width maximum 20%) of the operating parameters (error bars indicate the standard error) with second-order polynomial curve. The polynomial curve is only used to visualise the general trend, without any statistical significance.
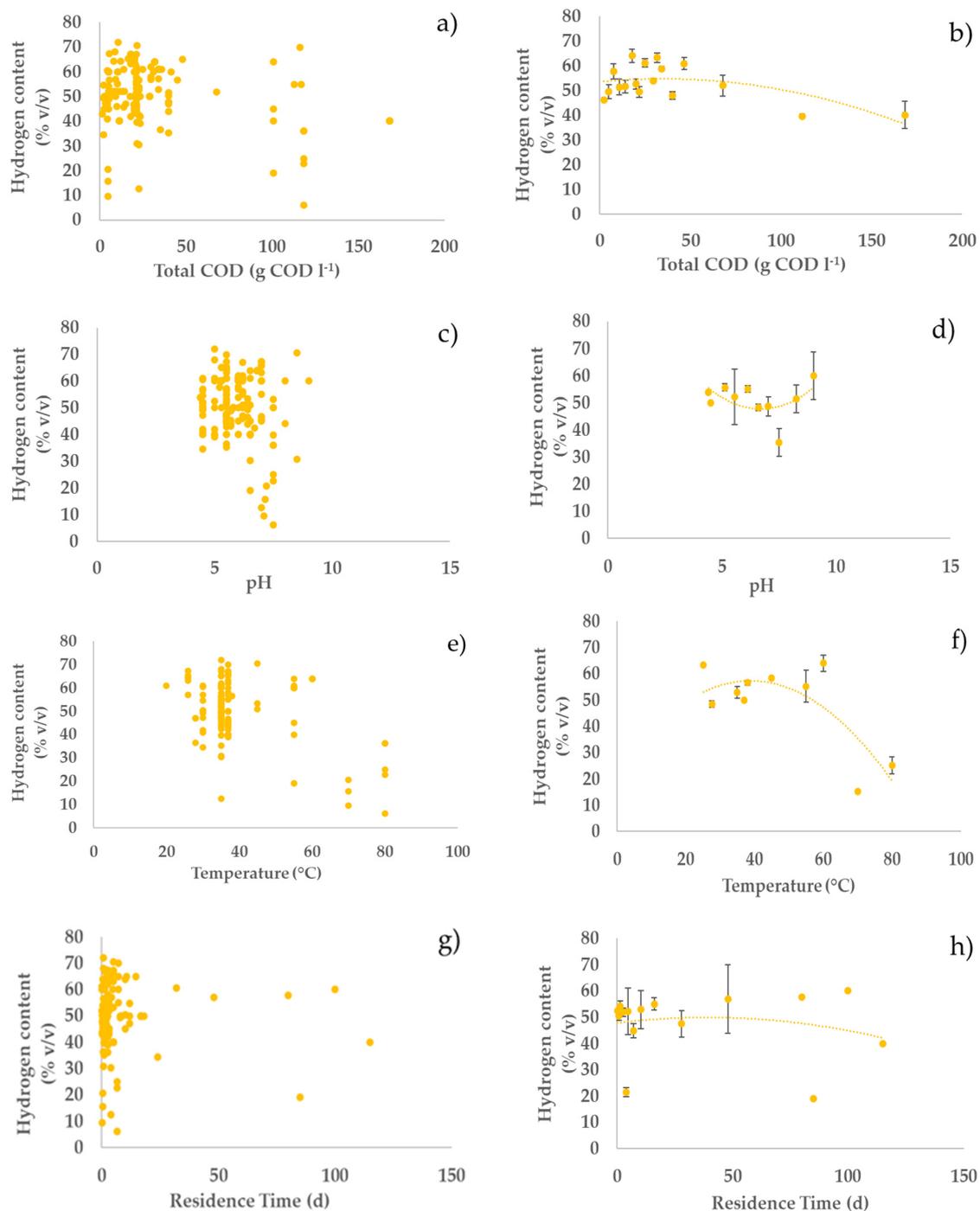
Qualitative observations that can be made from Figures 1–3 are as follows: The hydrogen yield (Figure 1) is relatively unaffected by substrate concentration in the feed and by the temperature. Additionally, it is negatively affected by higher pH values and higher residence time. The highest hydrogen yields (29% COD COD^{-1}) were reached with 4 g COD L^{-1} sucrose-rich wastewaters, under mesophilic conditions (39 °C), at pH 4.2, and

in continuous mode, with a hydraulic retention time of 0.50 d [20]. These experiments were carried out without the need for substrate pretreatment since they used simple substrates.

The hydrogen productivity (Figure 2) generally seems to increase as the feed concentration increases and is relatively unaffected by temperature. Generally, productivity undergoes a decreasing trend as the pH and the residence time increase; however, there are large variations in the literature data. The highest productivity (1422 mg L^{-1} d^{-1}) was achieved using glucose as substrate, under mesophilic conditions (35 °C), at acidic pH 5.7, and carried out in continuous mode, with a hydraulic retention time of 0.25 d [68].

The hydrogen content in biogas (Figure 3) seems relatively constant in a wide range of the operating parameters and is only negatively affected by large values of the substrate concentration, alkaline pH, and high temperature.

3.3. Descriptive Statistics and Performances

Table 3 shows mean and 90th percentile values for the process performance variables from the considered experimental data. The meaning of the 90th percentile values is that 10% of the considered experiments obtained yield, productivity, and content higher than the specified values.

Table 3. Mean, with standard error (in bracket), and 90th percentile values of process performance parameters.

Performance Parameter	Mean	90th Percentile
Hydrogen yield (% COD COD^{-1})	6.49 (0.27)	15.55
Hydrogen productivity (mg L^{-1} d^{-1})	135.49 (9.96)	305.16
Hydrogen content in biogas (% *v/v*)	51.00 (0.39)	64.00

The mean value of hydrogen yields (6.49% COD COD^{-1}) and hydrogen productivities (135 mg L^{-1} d^{-1}) were much lower than the 90th percentile value (15.55% COD COD^{-1}, 305.16 mg L^{-1} d^{-1}). This confirms what is observed in Figures 1 and 2—that the values of hydrogen yields and productivities are spread over a wide range in the literature. Only 10% of the considered studies obtained a hydrogen yield of 15.55% or higher and hydrogen productivity of 305.16 mg L^{-1} d^{-1} or higher. Only 2% and 6% of the experiments reached higher values of hydrogen yields (>20% COD COD^{-1}) and productivities (>500 mg l^{-1} d^{-1}), respectively. These results confirm the need for more studies to improve hydrogen production from wastewaters and organic waste. Unlike hydrogen yields and productivities, the hydrogen content does not vary considerably, as we noticed relatively close values between mean (51%), 90th percentile (64%), and highest value registered (72%).

In order to gain further insight on the effect of operating parameters on process performance, Table 4 shows the mean values of the operating parameters in the experiments that gave the best performance, i.e., in the experiments where the hydrogen yield, productivity, and content were at or above the 90th percentile. The mean operating parameters for the best experiments were compared with the mean operating parameters for all experiments.

Table 4. Mean values of operating parameters, with standard error (in bracket), for process performance parameters above 90th percentile compared with those of all experiments.

	All Experiments	Highest Hydrogen Yields (% COD COD^{-1})	Highest Hydrogen Productivities (mg L^{-1} d^{-1})	Highest Hydrogen Content (% *v/v*)
Substrate concentration (g COD L^{-1})	23.00 (1.32)	14.23 (3.04)	33.91 (4.22)	28.47 (5.92)
Residence time (d)	5.02 (0.53)	3.57 (0.74)	3.10 (1.00)	6.23 (0.64)
Temperature (°C)	37.98 (0.40)	40.05 (1.66)	37.98 (1.32)	39.91 (2.47)
pH	5.96 (0.05)	5.62 (0.13)	5.85 (0.12)	4.39 (0.77)

Compared with the parameters of all of the experiments, the highest hydrogen yields were obtained with lower substrate concentration (mean value 14.23 vs. 23 gCOD L^{-1}), shorter residence time (3.57 vs. 5.02 d), slightly lower pH (5.62 vs. 5.96), and a higher temperature (40.05 vs. 37.98 °C). The highest productivities were obtained with higher substrate concentrations (33.91 gCOD L^{-1}) and lower residence time (3.10 d). However, the mean temperature in the experiments with the best performance was similar to the mean temperature in all the experiments, indicating that this parameter had only a modest effect on process performance, at least in the considered experimental studies. The highest hydrogen content in the biogas is linked with a slightly lower substrate concentration than hydrogen productivities (28.47 mg L^{-1} d^{-1}), a longer residence time (6.23 d), a higher temperature (39.91 °C), and a lower pH (4.39).

3.4. Bivariate Analysis

The data failed the normality test (*p*-value < 0.05), which means that the data did not fit the normal distribution. Therefore, parametric tests could not be performed, and the data were analysed using non-parametric tests or bivariate analysis. Operational parameters were correlated with process performance parameters, and the relationship between tCOD, and production rate showed moderate strength (*p*-value < 0.05, τb = 0.238) using Kendal τb test. The correlation between the categorial operating parameters with process performance parameters showed that there is no significant difference (*p*-value < 0.05) between the different groups of the following parameters: pH (pHa, pHb, pHn), substrate composition (SS, SNS), operating mode (CN, BA), residence time (RT1, RT2, RT3), and substrate pretreatment (SY, SN).

3.5. Multi-Linear Regression Model

Afterwards, a general multi-linear fitting model with all the operational parameters was calculated for each process parameter.

3.5.1. Hydrogen Yield

Table 5 reveals the calculated correlation between hydrogen yields and the operating parameters of dark fermentation. Afterwards, the statistically insignificant terms (*p*-value > 0.05) were discarded, and the simplified model (Equation (2)) was calculated.

$$\text{Yield (\% COD COD}^{-1}) = 6.93 + 1.4 \times \text{pHa} - 1.05 \times \text{pHn} - 1.39 \times \text{pHb} + 1.2 \times \text{SS} - 1.19 \times \text{SNS} \qquad (2)$$

The operating parameters significantly affecting hydrogen yields are pH (pHa, pHn, pHb) and whether the substrate in the feed is soluble or non-soluble (SS, SNS). Acidic pH (pHa) has a positive correlation with hydrogen yield, unlike neutral and alkaline pH (pHn, pHb), which have a negative correlation. Hydrogen yield shows a positive correlation with soluble substrates (SS) and a negative correlation with non-soluble substrates (SNS). The model predicts an important effect of pH. For example, assuming a feed composed of soluble substrates, the model predicts a yield of 9.53% at acidic pH and only 6.2% at alkaline pH, confirming the effect of pH on the hydrogen yield already observed in Figure 1d.

Table 5. Correlation between hydrogen yields and operation parameters. Symbols annotation: Highly significant '***': $0 \leq p$-value < 0.001; fairly significant '**': $0.001 \leq p$-value < 0.01; significant '*': $0.01 \leq p$-value < 0.05; non-significant '•': $0.05 \leq p$-value < 0.1; highly non-significant'no symbol': p-value ≥ 0.1.

Parameters	Estimate	±SE	*t*-Value	*p*-Value
Intercept	6.93	0.36	19.48	$<2 \times 10^{-16}$ ***
pHa	1.4	0.53	2.65	0.008 **
pHn	−1.05	0.26	−4.01	6.20×10^{-5} ***
pHb	−1.93	0.67	−2.09	0.04 *
tCOD	−0.02	0.01	−1.93	0.05
SS	1.2	0.51	2.34	0.02 *
SNS	−1.19	0.27	−4.45	9.03×10^{-6} ***
Tm	−0.32	0.46	−0.67	0.49
Tt	−0.74	0.45	−1.66	0.98
CN	0.12	0.6	0.2	0.85
BA	−0.36	0.24	−1.47	0.14
RT1	−0.16	0.53	−0.3	0.76
RT2	−0.45	0.28	−1.61	0.11
RT3	−0.007	0.3	−0.025	0.98
IY	−0.64	0.48	−1.34	0.18
IN	0.05	0.32	0.16	0.87
SY	−0.81	0.6	−1.34	0.18
SN	−0.25	0.26	−0.95	0.34

3.5.2. Hydrogen Productivity

Table 6 shows the correlation between hydrogen productivity and operation parameters.

Table 6. Correlation between hydrogen productivity and operation parameters. Symbols annotation: Highly significant '***': $0 \leq p$-value < 0.001; fairly significant '**': $0.001 \leq p$-value < 0.01; significant '*': $0.01 \leq p$-value < 0.05; non-significant '•': $0.05 \leq p$-value < 0.1; highly non-significant 'no symbol': p-value ≥ 0.1.

Parameters	Estimate	±SE	*t*-Value	*p*-Value
Intercept	96.72	12.6	7.67	2.24×10^{-14} ***
pHa	64.13	18.7	3.43	6.12×10^{-4} ***
pHn	9.38	9.28	1.01	0.31
pHb	−28.76	23.67	−1.22	0.25
tCOD	1.69	0.36	4.75	2.14×10^{-6} ***
SS	107.34	18.16	5.91	3.73×10^{-9} ***
SNS	−21.09	9.49	−2.22	0.03 *
Tm	46.38	16.21	2.86	0.004 **
Tt	−11.83	15.88	−0.75	0.46
CN	174.52	21.08	8.28	$<2 \times 10^{-16}$ ***
BA	−13.13	8.6	−1.53	0.13
RT1	111.81	18.75	5.96	2.72×10^{-9} ***
RT2	−12.42	9.97	−1.25	0.21
RT3	−13.26	9.76	−1.36	0.17
IY	53.9	16.85	3.2	0.001 **
IN	−2.15	11.34	−0.19	0.85
SY	−5.5	21.13	−0.26	0.79
SN	29.87	8.59	3.48	5.14×10^{-4} ***

After the statistically insignificant terms (p-value > 0.05) were discarded, the simplified model (Equation (3)) was calculated.

$$\text{Productivity (mg L}^{-1}\,\text{d}^{-1}) = 96.72 + 64.13 \times \text{pHa} + 1.7 \times \text{tCOD} + 46.38 \times \text{Tm} + 107.34 \times \text{SS} - 21.09 \times \text{SNS} + 53.9 \times \text{IY} + 29.87 \times \text{SN} + 174.52 \times \text{CN} + 111.81 \times \text{RT1} \tag{3}$$

Acidic pH has a positive correlation with hydrogen productivity. Similarly, hydrogen productivity is also positively correlated with substrate concentration, mesophilic temperatures, soluble substrates in the feed, inhibition of methanogens, continuous mode, and short residence time (RT1). Insoluble substrates have negative effects on hydrogen productivity. The relatively large values of the coefficients for continuous flow and short residence time (174.52 and 111.81 d, respectively), indicate the large effect of these process parameters, which, for the residence time, is also evident from Figure 2h.

3.5.3. H_2 Content

Table 7 presents the correlation between hydrogen content and operation parameters.

Table 7. Correlation between hydrogen content and operation parameters. Symbols annotation: Highly significant '***': $0 \leq p$-value < 0.001; fairly significant '**': $0.001 \leq p$-value < 0.01; significant '*': $0.01 \leq p$-value < 0.05; non-significant '•': $0.05 \leq p$-value < 0.1; highly non-significant 'no symbol': p-value ≥ 0.1.

Parameters	Estimate	±SE	*t*-Value	*p*-Value
Intercept	53.96	1.16	46.23	$<2 \times 10^{-16}$ ***
pHa	−1.63	1.67	−0.97	0.33
pHn	−1.11	0.91	−1.23	0.22
pHb	−4.34	1.18	−3.67	0.0003 ***
tCOD	−0.09	0.03	−3.48	0.0005 ***
SS	−2.93	1.65	−1.78	0.07 •
SNS	−1.22	0.88	−1.38	0.17
Tm	−1.78	1.50	−1.18	0.24
Tt	−3.71	1.26	−2.94	0.003 **
CN	3.00	2.92	1.03	0.30
BA	−0.91	1.48	−0.61	0.54
RT1	−1.71	1.66	−1.03	0.31
RT2	−1.94	0.94	−2.06	0.04 *
RT3	−1.13	0.97	−1.17	0.24
IY	−3.09	1.50	−2.05	0.04 *
IN	−0.17	1.24	−0.14	0.89
SY	−5.49	2.07	−2.65	0.008 **
SN	−0.12	0.80	−0.15	0.88

After the statistically insignificant terms ($p > 0.05$) were discarded, the simplified model (Equation (4)) was calculated.

$$\text{Hydrogen content } (\% \; v/v) = 53.96 - 4.34 \times \text{pHb} - 0.09 \times \text{tCOD} - 3.71 \times \text{Tt} - 5.49 \, \text{SY} \quad (4)$$

Hydrogen content is significantly negatively correlated with alkaline pH (pHb), substrate concentration (tCOD), thermophilic temperature (Tt), and substrate pretreatment (SY). However, comparing the coefficients in Equation (4) with the coefficients in Equations (2) and (3), it is evident that the effect of the operating parameters on hydrogen content is modest, compared with their effect on yield and productivity. For example, assuming a feed of 10 gCOD L^{-1}, with mesophilic conditions and no pretreatment of the substrate, the model predicts that alkaline pH reduces the hydrogen content in the biogas only from 53.06% (at acidic or neutral pH) to 48.72%. The model, therefore, confirms the evidence from Figure 3 that process parameters have relatively little influence on the hydrogen content in the biogas.

4. General Discussion

In this section, the findings of this study are compared with other studies in the literature (Section 4.1), and the implications of this study for process optimisation are discussed (Section 4.2).

4.1. Comparison with Other Studies in the Literature

Various studies have investigated the effect of different operating parameters on hydrogen production, focusing on one or two operating parameters. The results were not widely reproducible due to the range of operating conditions, the nature of the inoculum, and the substrate used. Therefore, the importance of our study is to combine and analyse the results of previous studies to identify general trends and optimal factors affecting hydrogen production.

Previous studies have highlighted the importance of pH on the performance of dark fermentation and hydrogen production. The pH can affect the metabolic pathway of fermentative bacteria, especially hydrogen-producing bacteria, and influence hydrogenase activity [69,70]. While fermentation of organic compounds occurs at a pH range between 4 and 9, hydrogen-consuming bacteria or methanogens have an optimal pH range between 6.8 and 8 [71,72]. This can explain the finding of our analysis which indicates that acidic pH is the most favourable for hydrogen production with dark fermentation.

Regarding the temperature, most of the studies in the literature on dark fermentation have used mesophilic conditions. Thermophilic conditions have many disadvantages over microbial growth which will negatively affect hydrogen production. The increase in temperature involves cellular growth inhibition, thermal denaturation of proteins especially enzymes, decrease in extracellular polymeric substances, and most of all increase in energy cost [73]. However, our analysis shows that, although there is a rather weak positive effect of mesophilic conditions, hydrogen production with high yield is also possible under thermophilic conditions, e.g., high hydrogen yields (20 and 21% COD COD^{-1}) were obtained at 70 and 60 °C, respectively, under neutral pH (Figures 1–3; parts e,f).

Concerning the residence time, Liu et al. observed a decrease in gas (hydrogen and methane) production during AD of organic household solid waste, by thermophilic microorganisms at 70 °C, when the residence time was decreased from 6 d to 1 d [74]. Furthermore, hydrogen production yields were higher at shorter HRT (2 h), in continuous mode using a concentrated substrate, slightly acidic pH, and high temperature (55 °C) [13]. Similarly, an increase in hydrogen production rate was observed when HRT decreased from 8 h to 1 h [75]. An optimal HRT of 2 h resulted in the highest hydrogen production during the AD of cassava wastewater in an anaerobic fluidised bed reactor at 28 °C. On the other hand, Ziara et al. achieved the highest hydrogen yields (0.85 mol H$_2$/mol lactate) by fermenting lactate wastewater at 45 °C for 180 h, with an initial pH of 8.5 [39]. Additionally, this study confirms the impact of short residence time on hydrogen yields, productivity, and content (Figures 1–3; parts g,h). However, one experiment conducted by Yu et al. noted an increase in hydrogen yield, from 1.74 mol H$_2$/mol hexose to 2.14 mol H$_2$/mol hexose, when HRT increased from 2 h to 24 h [13]. The outcomes depend on the nature of substrate, its solubility, availability, and on the nature of the inoculum. To further improve hydrogen yields from the AD of organic waste and wastewaters, it is important to take into consideration all the parameters discussed above as complementary and not individual parameters.

4.2. Process Considerations

Although the performance of a hydrogen production process with DF will be highly dependent on the specific feedstock used, this study offers some general insight on how to optimise process parameters to maximise process performance. Generally, higher substrate concentration in the feedstock should be preferred, as it tends to give higher hydrogen productivities with a relatively small impact on hydrogen yield and content in the biogas. Acidic pH values should be preferred over neutral and alkaline values. Indeed, acidic pH values generally tend to give higher yields, with relatively little, but still positive effects on hydrogen productivity and content. pH control to acidic values seems an interesting strategy to maximise hydrogen production by reducing the conversion of hydrogen into methane. It is interesting to observe that DF naturally tends to be acidic due to the production of SCOAs; therefore, it is likely that an acidic pH can be maintained without the addition of pH balancing chemicals, which increases the attractiveness and environmental sustainability of this process. As far as the temperature is concerned, our analysis indicates that hydrogen production using AD is relatively unaffected by temperature in a wide range, 30–60 °C, with some indication of a slightly better performance at the lowest end of this range. From the process point of view, this indicates that mesophilic conditions should generally be preferred to thermophilic conditions, because of the lower energy consumption to maintain the temperature of the digester. Our analysis indicates that residence time

should be maintained at relatively low values to maximise hydrogen production. Indeed, low residence time contributes to washing out the methanogens, which, in turn, contributes to increasing hydrogen yield. Notably, low residence time means smaller volumes of the reactors, which has a direct positive impact on the volumetric productivity, as observed in our analysis. Furthermore, smaller reactors also contribute to decreasing the capital and operating costs of the process.

Another process variable that tends to favour hydrogen production is the nature of the feedstock, with soluble substrates tending to give higher yield and productivity than insoluble substrates. Overall, the pretreatment of the substrate and the inhibition of methanogenesis seem to have only a modest effect on the process. It is interesting to observe that continuous fermentation seems to have a relatively large positive effect on productivity.

The most favourable conditions for hydrogen production identified in this analysis—namely, high substrate concentration, acidic pH, and short residence time—should be targets of more research effort to optimise the process for full-scale commercialisation. Clearly, the economics and sustainability of the process should also be investigated. On the other hand, it is worth noting that only less than 3% of the considered studies investigated hydrogen production from municipal wastewaters. Although municipal wastewaters are typically diluted, their conventional aerobic treatment is energy intensive, and an anaerobic process with hydrogen production in the first stage and methane production in the second stage would represent an interesting alternative to current processes. This also deserves further investigation.

5. Conclusions

A large volume of literature is being published on the use of DF for hydrogen production. This study identified the general effects of operating parameters on the process performance of dark fermentation, which was characterised by the hydrogen yield, productivity, and content in the biogas. Although the results in the literature were highly scattered due to the different feedstocks and operating conditions used, generally, the best process performance was obtained at relatively acidic pH, short residence time, and high substrate concentration. Soluble substrate and continuous operating mode also generally gave better process performance. Working under optimised conditions can significantly enhance hydrogen production, and more studies on process optimisation are required for the successful commercialisation of this process.

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