Biomass and Bioenergy 110 (2018) 75–79

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com/locate/biombioe

Short communication

Influence of white-rot fungus *Polyporus brumalis* BRFM 985 culture conditions on the pretreatment efficiency for anaerobic digestion of wheat straw

Elsa Rouches[a], Simeng Zhou[b], Michelle Sergent[c], Sana Raouche[b], Helene Carrere[a,*]

[a] LBE, Univ Montpellier, INRA, 102 Avenue des Etangs, 11100 Narbonne, France
[b] Aix Marseille Univ, INRA, BBF, Biodiversité et Biotechnologie Fongiques, Marseille, France
[c] Aix Marseille Univ, LISA EA4672, Campus scientifique de Saint Jérôme, 13397, Marseille, France

ARTICLE INFO

Keywords:
Biogas
Fungal pretreatment
Lignocellulosic biomass
Solid state fermentation
Surface response methodology

ABSTRACT

For the first time, *Polyporus brumalis* BRFM985 was cultivated on wheat straw to investigate the simultaneous effects of pretreatment parameters on anaerobic digestion: these include initial substrate humidity, temperature, duration, and metal supplementation. Surface response methodology was applied to quantify the importance of each parameter, as well as the synergistic effects between them. Firstly, metal addition and secondly, pretreatment duration, both resulted in a positive impact. According to calculations, the highest methane production (182 dm^3 of methane per kilogram of initial Total Solids) is associated to pretreatment with metal addition during 20 days. In comparison with the least optimal conditions (118 $dm^3.kg^{-1}$ without metal addition, during 15 days), this result implies a 52% increase.

1. Introduction

Despite the advantages that biogas production from crop residues may display [1], its economic competitiveness still needs to be improved, notably by applying low-cost pretreatments. The main objective of these pretreatments implies lignin degradation, which enhances methane production during anaerobic digestion. Even though diverse pretreatments have shown to be efficient at laboratory scales, their industrial applications are often restricted by excessive costs. Investigations on white-rot fungi pretreatments for anaerobic digestion are still scarce, but they have pointed to this procedure as efficient, low-cost and environmentally-friendly [2,3].

After an initial screening step [4], the *Polyporus brumalis*_BRFM 985 strain was found to be efficient in the pretreatment of wheat straw for anaerobic digestion [5]. Fungal pretreatment efficiency not only depends on the substrate and fungal strain, but also on the culture conditions [2,3]. For example the pretreatment of wheat straw by *Polyporus brumalis*-BRFM 985 for ethanol production was investigated by Zhou and al [6]. Depending on fungal pretreatment parameters, the authors observed net cellulose enzymatic hydrolysis yields ranging between 28% and 132% relative to the control value (without fungal pretreatment).

Studies investigating fungal pretreatment parameters generally evaluate the efficiency by measuring cellulose hydrolysis or lignin losses [3]. This is, however, not sufficient for their impact on anaerobic digestion to be assessed. Indeed, the hydrolysis of hemicelluloses by an efficient hemicellulase cocktail must also be taken into account. Fungal biomass can also be converted into biogas. Moreover, the evaluation of pretreatment efficiency most often does not account for mass losses. Inadequate pretreatment conditions can lead to a decrease in methane production. In general, pretreatments for anaerobic digestion have been performed under fixed culture conditions, either on several fungal strains with a specific lignocellulosic substrate or with a specific strain on several lignocellulosic substrates [2]. Very few studies have investigated process parameters such as moisture content [7,8] or duration [9]. Consequently, the optimization of fungal pretreatment parameters for methane production still requires further research efforts. A concomitant investigation of process parameters could lead to the identification of potential synergy effects and to the classification of their order of significance for methane production.

In the present study, Surface Response Methodology was applied to investigate the influence of culture parameters on *P. brumalis*_BRFM 985 pretreatment efficiency for enhancing biogas production from wheat straw anaerobic digestion.

Abbreviations: BRFM, Bank of Fungal Resources of Marseille; CIRM, International Center of Microbial Resources; RSM, Response Surface Methodology; TS, Total Solids; WRF, White-Rot Fungi; WW, Wet Weight

* Corresponding author.
E-mail address: helene.carrere@inra.fr (H. Carrere).

https://doi.org/10.1016/j.biombioe.2018.01.018
Received 24 October 2017; Received in revised form 23 January 2018; Accepted 23 January 2018

Table 1
Experimental design with Doehlert design and responses measured. Coded variables for experimental points, culture parameters, dry mass losses and methane production of pretreated and control straws (*Minimum* and **maximum** response values).

Samples	Coded variables				Real variables: culture parameters				Responses measured		
	X1 (Metals)	X2 (Duration)	X3 (Temperature)	X4 (WW/TSi)	Metals	Duration (d)	Temperature *(°C)*	WW/ TS initial	CH_4 production at day 6 ($dm^3.kg^{-1}$)	CH_4 production at day 57 ($dm^3.kg^{-1}$)	Dry Mass Losses *(%)*
1	1	0.5	0	0.866	Yes	17.5	25	4.5	71 ± 12	164 ± 7	18.9 ± 1.3
2	1	−0.5	0	−0.866	Yes	12.5	25	2.1	41 ± 8	129 ± 14	12.9 ± 1.3
3	1	0.5	0	−0.866	Yes	17.5	25	2.1	54 ± 1	144 ± 7	18 ± 0.1
4	1	−0.5	0	0.866	Yes	12.5	25	4.5	71 ± 4	168 ± 11	15.4 ± 0.8
5	1	0.5	0.8165	0.2887	Yes	17.5	30	3.7	**82** ± 2	173 ± 5	**25.0** ± **0.4**
6	1	−0.5	−0.8165	−0.2887	Yes	12.5	20	2.9	50 ± 5	125 ± 23	5.1 ± 1.2
7	1	0.5	−0.8165	−0.2887	Yes	17.5	20	2.9	48 ± 9	151 ± 24	12.4 ± 1.4
8	1	0.0	−0.8165	0.5774	Yes	15	20	4.1	49 ± 1	118 ± 2	9.1 ± 0.6
9	1	−0.5	0.8165	0.2887	Yes	12.5	30	3.7	74 ± 3	**176** ± **16**	18.2 ± 0.5
10	1	0.0	0	0	Yes	15	25	3.3	69 ± 5	152 ± 26	23.1 ± 0.4
14	−1	1.0	0	0	No	20	25	3.3	56 ± 7	140 ± 37	24.2 ± 0.5
15	−1	−1.0	0	0	No	10	25	3.3	48 ± 4	152 ± 25	12.6 ± 1.1
16	−1	0.5	0	0.866	No	17.5	25	4.5	58 ± 4	129 ± 6	20.0 ± 0.9
17	−1	−0.5	0	−0.866	No	12.5	25	2.1	39 ± 4	112 ± 12	11.1 ± 0.3
18	−1	0.5	−0.8165	−0.2887	No	17.5	20	2.9	51 ± 1	129 ± 8	8.1 ± 1.0
19	−1	0.0	−0.8165	0.5774	No	15	20	4.1	47 ± 4	120 ± 8	2.6 ± 0.2
20	−1	−0.5	0.8165	0.2887	No	12.5	30	3.7	59 ± 2	125 ± 4	*0.7* ± *0.7*
21	−1	0.0	0.8165	−0.5774	No	15	30	2.5	54 ± 5	*111* ± 10	1.7 ± 1.5
11	1	0.0	0	0	Yes	15	25	3.3	93 ± 5	242 ± 9	22.9 ± 0.7
12	1	0.0	0	0	Yes	15	25	3.3	90 ± 9	222 ± 15	21.7 ± 1.5
13	1	0.0	0	0	Yes	15	25	3.3	88 ± 12	208 ± 28	21.3 ± 1.0

Table 2
Extrema and coefficients for response surface of methane production at days 6 and 57.

Anaerobic digestion duration (d)	Metals	Time (d)	WW/ TS initial	Temperature (°C)	Calculated CH_4 yield ($dm^3.kg^{-1}$)	
					Maximum	Minimum
6	Yes	15.85	4.15	29.67	86	
		17.12	3.47	19.62		47
	No	16.29	4.06	29.79	72	
		17.36	3.91	20.39		45
	$Y = 66.23 – 2.79X_1 + 4.76X_2 + 9.74X_3 + 10.30X_4 - 11.79X_2^2 - 9.87X_3^2$ $- 6.88X_4^2 + 0.25X_1X_2 - 3.14X_1X_3 - 3.19X_1X_4$ $- 8.36X_2X_3 + 9.21X_2X_4 + 12.43X_3X_4$ p-value (F-test): $3.19*10^{-4}$					
57	Yes	11.67	4.02	28.22	180	
		19.84	3.46	26.36	182	
		13.92	3.12	19.19		121
	No	10.32	3.76	25.78	152	
		19.86	3.15	23.73	144	
		15.22	4.07	19.98		118
	$Y = 139.19 – 13.09X_1 + 2.57\ X_2 + 11.34X_3 + 8.39X_4 + 19.56X_2^2$ $- 7.88X_3^2 - 9.32X_4^2 - 5.53X_1X_2 - 3.15X_1X_3 - 11.65X_1X_4 - 11.33X_2X_3$ $- 7.60X_2X_4 + 14.45X_3X_4$ p-value (F-test): $7.22*10^{-2}$					

2. Material and methods

2.1. Experimental design

2.1.1. Construction of the experimental design

Four factors characterise the experimental design [6]: pretreatment duration (X_2), culture temperature (X_3), initial wheat straw humidity expressed as a Wet Weight to initial Total Solids ratio (WW/TS initial, X_4), and addition or not of a metal solution (X_1) (Table 1). To estimate the coefficients and to ensure a good prediction quality for the experimental domain, a D-optimal design was built with 18 distinct points (Table 1). Experiments 11 to 13 were performed at different periods and were used for estimating the experimental variance.

2.1.2. Analysis of the experimental design and response variables

As the 'Metal' factor is qualitative and comprises two levels (Yes or No), a specific model was built (Table 2). The model coefficients were estimated by least squares regression [11]. R software (version 3.2.1) and its dedicated 'rsm' library were employed to determine response surfaces for CH_4 production after 6 days and 57 days. Regression model validity was estimated using a Fischer F-test with a 90% confidence level at least for comparing the variance explained by the model with the global residual variance. To ensure the robustness of the RSM, global residuals took into account model residues and the variance of the experimental response for samples 11 to 13. Extrema of response surfaces within the experimental domain and corresponding culture parameters were computed using model equations and Excel solver.

2.2. Fungal pretreatment for anaerobic digestion

2.2.1. Biological materials and solid state fermentation

The complete methodology has been described by Zhou et al. [6]. In summary, the *Polyporus brumalis*_BRFM 985 strain was provided by the "Centre International de Ressources Microbiennes" (CIRM- CF; https://www6.inra.fr/cirm_eng/CIRM-CF), hosted by the National Institute of Agricultural Research (INRA), Marseille, France. *P. brumalis* was cultivated on winter wheat straw (*Triticum aestivum*, Haussmann). The straw was harvested in July 2012 (sowed in October 2011) in the North of France (4°13′54,5″E, 48°50′18″N) and provided by Vivescia (Reims, France); it was dried naturally, stored in a sheltered area and chopped (≈4 mm length). Dry mass fractions of polymers were determined by NREL method [10] and resulted in 37.5% cellulose, 27.5% hemicelluloses and 23.0% lignin.

Solid State Fermentation was performed in 250 mL packed-bed columns. For each column 20 g TS of chopped straw were humidified with 9 cm^3 deionized water and sterilized at 110 °C for 30 min in an autoclave bag. After cooling to room temperature, 10 cm^3 of fungal inoculum suspension (preparation protocol is detailed in Ref. [6]) were added to the bags with the required amount of sterilized water and 1 cm^3 of sterile metal solution (only for certain samples). The metal solution was composed of $MnSO_4$; $CuSO_4$ and $FeSO_4$ (18 mmol.dm^{-3}