

## THERMOPHILIC DARK FERMENTATION OF SOYBEAN MOLASSES: IS TEMPERATURE OPTIMIZATION THE KEY TO MAXIMIZING THE H<sub>2</sub> PRODUCTION?

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### ABSTRACT

Soybean molasses is a low-value by-product of the soybean biorefinery with an elevated annual production. One strategy to improve this by-product value is utilizing it as a substrate for dark fermentation to produce hydrogen as an energy. Three 3-liter Sequencing Batch Biofilm Reactors (AnSBBRs) were run at 50, 55, and 60 °C with three applied organic loading rates (OLRA) of 16, 21, and 26 kgCOD/m<sup>3</sup>/d to assess the performance of dark fermentation process. Optimizing H<sub>2</sub> production was found to depend critically on operational temperature. Comparing the lowest temperature operation (50 °C) to those at 55 °C and 60 °C revealed an increase in molar productivity of 46 and 247%, respectively. The systems operating at 50 °C and 26 kgCOD/m<sup>3</sup>/d achieved the highest performance with productivity of 51.4 mol/m<sup>3</sup>/d, a biogas quality of 65 % in hydrogen, and total volatile acids concentration in effluent of 1733 mgHAc/L. This improved performance may be due to the predominance of acetic acid and butyric acid pathways and the low occurrence of propionate and lactate fermentations.

**Keywords:** Temperature influence. Agro-industrial wastewater. Kinetic modeling. Mixed-culture fermentations.

## 1 INTRODUCTION

Soybean molasses is a dark syrupy by-product of the protein concentrate production process resulting from the aqueous alcohol extraction of soybean seeds. This by-product has low commercial value, and its typical application is as alternative animal feed. Due to the high amount of soybean molasses produced annually (estimated at 100,000 tons globally), large quantities accumulate, and the portion not utilized by industries or agricultural sectors is disposed of as liquid manure or in landfills, probably causing severe ecological problems<sup>1,2</sup>.

The world needs energy, and hydrogen is a prominent candidate as a future fuel. Biological processes (photo-fermentation, dark fermentation, and biophotolysis) emerge as potential routes for H<sub>2</sub> production. Dark fermentation has the advantage of being light-independent and has a high production rate. Furthermore, dark fermentation can utilize by-products or waste streams as substrates and convert them into energy (H<sub>2</sub>), improving stream market value<sup>3</sup>.

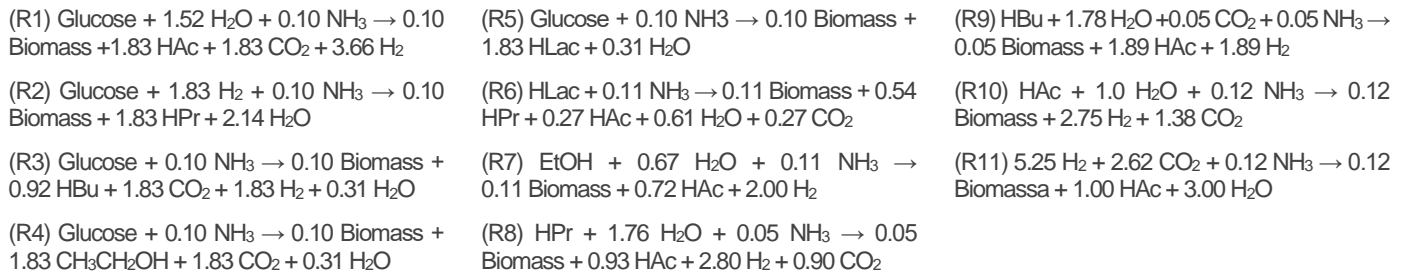
In this context, soybean molasses is a potential substrate for the dark fermentation process once it contains approximately 30 – 40 % of total sugars<sup>1</sup>. Therefore, this study evaluates the H<sub>2</sub> production during the dark fermentation of soybean molasses as a by-product of soybean processing, varying the temperature in the thermophilic range and the applied organic loading rate (OLRA).

## 2 MATERIAL & METHODS

Three Sequencing Batch Biofilm Reactors (AnSBBRs) with liquid phase recirculation (cycle length of 3.0 h, filling time of 1.5 h, working volume of 3.0 L, and feeding/discharge of 1.0 L) were operated at thermophilic temperatures of 50 °C, 55 °C and 60 °C and designated as systems S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> respectively. The wastewater was based on soybean molasses and supplemented with sodium bicarbonate (0 – 0.03 gNaHCO<sub>3</sub>/gCOD) and a micronutrient solution<sup>4</sup>. The reactor's inoculum was sludge from a UASB sugarcane stillage wastewater treatment in São Paulo (Brazil) with total solids of 26.9 g/L and volatile solids of 16.5 g/L.

Thermophilic reactors start-up followed the strategy proposed by Lovato et al.<sup>9</sup> in which there were a ramp of temperature (30 °C to temperature operation, 5 °C/d rate) and a ramp of influent concentration (1 gCOD/L to desired concentration, 1 gCOD/L/d rate). After the start-up period, three OLR<sub>A</sub> (16, 21, and 26 kgCOD/m<sup>3</sup>/d) were applied to each reactor. Between each OLR<sub>A</sub> change, a ramp of influent concentration at 1 gCOD/L/d rate was conducted.

The analyses followed the Standard Methods for Water and Wastewater Examination<sup>5</sup>. Intermediate compounds of anaerobic metabolism (ethanol and acetic, propionic, and butyric acid) and biogas composition (H<sub>2</sub>, CH<sub>4</sub>, and CO<sub>2</sub>) were analyzed by gas chromatography<sup>4</sup>. Lactic acid concentration was measured by the Borshevsckaya et al.<sup>6</sup> spectrophotometric method. A kinetic model based on Angelidaki et al.<sup>7</sup> and presented by Augusto et al.<sup>8</sup> modeled the experimental data obtained along a cycle of the assays S<sub>1</sub>-26, S<sub>2</sub>-26, and S<sub>3</sub>-26 considering the reaction of Equations R1 to R11.



### 3 RESULTS & DISCUSSION

Table 1 contains the monitored variables and performance indicators for nine assays (three assays for each temperature).

**Table 1** Monitored variables and performance indicators for all assays

Parameter	S <sub>1</sub> -16	S <sub>1</sub> -21	S <sub>1</sub> -26	S <sub>2</sub> -16	S <sub>2</sub> -21	S <sub>2</sub> -26	S <sub>3</sub> -16	S <sub>3</sub> -21	S <sub>3</sub> -26
OLR <sub>A,COD</sub>	16.7	22.0	27.2	16.0	21.9	26.8	16.3	22.0	26.5
ε <sub>COD</sub> (%)	29 ± 4	30 ± 3	24 ± 3	23 ± 3	22 ± 2	22 ± 3	23 ± 2	22 ± 2	20 ± 3
OLR <sub>A,carb</sub>	6.6	8.6	11.1	6.5	8.7	10.8	6.5	9.5	11.2
ε <sub>carb</sub> (%)	76 ± 7	85 ± 4	77 ± 5	56 ± 4	44 ± 2	38 ± 3	46 ± 3	42 ± 3	33 ± 5
TVA <sub>INF</sub>	442 ± 25	585 ± 19	767 ± 22	419 ± 9	617 ± 14	753 ± 18	446 ± 32	609 ± 14	745 ± 19
TVA <sub>EFF</sub>	1058 ± 85	1700 ± 36	1733 ± 71	1045 ± 64	1139 ± 62	1300 ± 62	851 ± 83	905 ± 80	1142 ± 91
pH <sub>EFF</sub>	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1
V <sub>H<sub>2</sub></sub>	200 ± 19	215 ± 27	418 ± 52	138 ± 14	239 ± 26	280 ± 21	59 ± 6	125 ± 11	115 ± 10
X <sub>H<sub>2</sub></sub> (%)	43 ± 6	56 ± 5	65 ± 3	52 ± 5	68 ± 5	65 ± 6	51 ± 2	55 ± 2	63 ± 6
PrH <sub>2</sub>	25.0	27.1	51.4	17.6	29.8	35.0	7.5	15.9	14.8

Notation: Carb – carbohydrates; OLR<sub>A</sub> – applied organic loading rate (kgCOD/m<sup>3</sup>/d or kgCarb/m<sup>3</sup>/d); ε – organic matter removal efficiency; INF – influent; EFF – effluent; TVA – total volatile acids (mgHAc/L); V<sub>H<sub>2</sub></sub> – volume of hydrogen generated per cycle (NmL/cycle); X<sub>H<sub>2</sub></sub> – hydrogen content in the biogas; PrH<sub>2</sub> – molar hydrogen productivity (mol/m<sup>3</sup>/d).

Organic matter removal efficiency on a COD basis varied between 20 and 30 %, which is expected once process operational conditions successfully inhibit methanogenic activity (there was no detection of CH<sub>4</sub> in any assay). The values of pH<sub>EFF</sub> for all assays fluctuated between 4.5 and 4.6, an adequate value for hydrogenogenic reactors.

Regarding carbohydrate removal, efficiency values ranged from 76 – 85 %, 38 – 56 %, and 33 – 46 % for systems S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub>, respectively, considering the three OLR<sub>A</sub> (16, 21, and 26 kgCOD/m<sup>3</sup>/d). These results suggest that the rising temperature had a detrimental effect on carbohydrates consumption. Temperature increases also negatively affect volatile acids production. For the highest OLR<sub>A</sub> (26 kgCOD/m<sup>3</sup>/d), the systems S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> achieved TVA concentrations of 1733 ± 71, 1300 ± 62 and 1142 ± 91 mgHAc/L, respectively. So, increasing temperature decreases the effectiveness of these systems in degrading carbohydrates into volatile acids.

The hydrogen content in biogas remained between 63 % and 65 % for the highest OLR<sub>A</sub> (26 kgCOD/m<sup>3</sup>/d) of systems S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub>, the volume of hydrogen produced per cycle decreased from 418 to 280 and 115 NmL/cycle when the temperature increased from 50 °C to 55 °C and 60 °C, respectively.

Regarding volumetric productivity, raising the OLR<sub>A</sub> for systems S<sub>1</sub> (50 °C) and S<sub>2</sub> (55 °C) resulted in a constant improvement in volumetric production, with values of 51.4 and 35.0 mol/m<sup>3</sup>/d, respectively. In system S<sub>3</sub> (60 °C), productivity increased from 7.5 to 15.9 mol/m<sup>3</sup>/d when OLR<sub>A</sub> was increased from 15 to 21 kgCOD/m<sup>3</sup>/d, but then decreased to 4.8 mol/m<sup>3</sup>/d when OLR<sub>A</sub> was raised from 21 to 26 kgCOD/m<sup>3</sup>/d.

Figure 1 shows the consumption of carbohydrates from soybean molasses for systems S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> at OLR<sub>A</sub> 26 kgCOD/m<sup>3</sup>/d. System S<sub>1</sub> (50 °C) presented a predominance of acidogenesis of acetic acid (R1) and butyric acid (R3), indicating an acetate-butyrate fermentation associated with higher H<sub>2</sub> production. Also, in system S<sub>1</sub>, glucose consumption via propionate (R2) and lactate (R5) pathways was negligible (routes of consumption and non-production of hydrogen). System S<sub>2</sub> (55 °C) had butyric acid (R3) and ethanol (R4) production as the main acidogenesis pathways, and lactic acid production (R5) became relevant. Regarding system S<sub>3</sub> (60 °C), glucose consumption was lower than in systems S<sub>1</sub> (50 °C) and S<sub>2</sub> (55 °C). Furthermore, acetic acid production (R1) was minimal, and the propionate (R2) and lactate (R5) acidogenesis pathways shared more than one-third of the glucose consumed, explaining the low performance of the reactor at 60 °C.



Figure 1 Flowchart of consumption of glucose for each metabolic pathway based on kinetic model data

## 4 CONCLUSION

Soybean molasses thermophilic dark fermentation was revealed to be a promising process for producing hydrogen gas. When compared to 55 °C and 60 °C, respectively, the lowest temperature operation (50 °C) presented a 46 and 247% increase in molar productivity. The systems operating at 50 °C and 26 kgCOD/m<sup>3</sup>/d achieved the highest performance with productivity of 51.4 mol/m<sup>3</sup>/d, a biogas quality of 65 % in hydrogen, and total volatile acids concentration in effluent of 1733 mgHAc/L. This improved performance may be due to the predominance of acetic acid and butyric acid pathways associated with high hydrogen production. Also, the low occurrence of propionate and lactate fermentation pathways with H<sub>2</sub> consumption and H<sub>2</sub> non-production contributed to system efficiency. The poor performance of the reactor at 60 °C may be due to the low carbohydrate consumption and significant occurrence of undesirable pathways (propionic and lactate acidogenesis) that shared more than one-third of the glucose consumed.

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