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Effect of temperature on biohydrogen and biomethane production using a biochemical potential test with different mixtures of sewage sludge, vinasse and poultry manure

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ABSTRACT

A two-stage anaerobic co-digestion system has been proposed, comprising a first acidogenic stage at different temperatures, where biohydrogen is obtained; and a second mesophilic methanogenic stage where biomethane is obtained. The objective of this research was to evaluate the biochemical hydrogen potentials (BHP) at different temperature ranges, and their effect on the biochemical methane potentials subsequently carried out with the effluents from the BHP, in batch trials. Also, to evaluate the effect of adding a third co-substrate (poultry manure) to the mixture of sewage sludge and wine vinasse.

For the BHP tests, temperatures of 35°, 55° and 70 °C were tested in mixtures of sewage sludge:vinasse (50:50) and sewage sludge:vinasse:poultry manure (49.5:49.5:1). It was found that the addition of poultry manure and a thermophilic temperature of 55 °C was ideal for biohydrogen generation with the highest recorded yield of 27.1mLH_2/gVS.

In the BMP trials consisting of effluent from the BHP and programmed at 35 °C, it was found that the effluent from the hyperthermophilic BHP trials (70 °C) generated more biogas and had a higher methane yield (117.36 mLCH_4/gVS), and that this yield was higher for the sewage sludge and vinasse mixture alone. This proportion also had the highest percentage of VS removal (45.74%). The Modified Gompertz model was the best fit to the experimental data, with $R^2 > 0.983$ in all cases.

The search for the most suitable temperature ranges for the production of H_2 and CH_4 is necessary in order to be able to efficiently realise this technology on a larger scale.

1. Introduction

Current population growth is resulting in major environmental problems related to energy demand and the abuse of fossil fuels, the accumulation of organic waste and greenhouse gas emissions (Abdur Rawoof et al., 2021; El Ibrahimi et al., 2021). Another consequence is seen in the exponentially increasing number of wastewater treatment plants, which is leading to the generation of large quantities of sewage sludge that are difficult and costly for these plants to treat (Zhang et al., 2016). Sewage sludge consists of a mixture of solids (suspended or dissolved substances) and a high percentage of water (>95%) (Zhang et al., 2021). Although their composition varies depending on the pollutant load, these biosolids usually contain large amounts of oxidisable organic matter, suspended solids, pathogens, toxic substances (heavy metals and organic pollutants) and nutrients (mainly nitrogen and phosphorus) (Borowski et al., 2014; Venegas et al., 2021).

The wine sector is one of the most important within the Spanish agri-food industry, especially in Andalusia and, more specifically, in the Jerez de la Frontera region. This industry generates large volumes of wastewater rich in dissolved organic matter (35–40 g COD/L) (Marie et al., 2019). This wastewater is called vinasse when it comes from the distillation of wine to obtain brandy de Jerez. Vinasse is an acid effluent (pH around 3.5) consisting of organic and inorganic compounds such as acetic acid, glycerol, lactic acid, ethanol, potassium, nitrogen, phosphates, calcium and sulphate (Sillero et al., 2022a; Tena et al., 2021a). The highly polluting organic matter in the form of soluble sugars and phenols means that vinasse is considered an environmental hazard if not properly managed (Cabrera Díaz and Díaz Marrero, 2013; Djalma Nunes Ferraz Júnior et al., 2014; Cremonez et al., 2021).

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Abbreviations

SS	Sewage Sludge
V	Vinasse
PM	Poultry manure
TPAcD	Temperature-Phase Anaerobic co-Digestion
TCOD	Total chemical oxygen demand, in milligrams per liter
SCOD	Soluble chemical oxygen demand, in milligrams per liter
TVFA	Total volatile fatty acids, in milligrams acetic equivalent per liter
TS	Total solids, in grams per liter
VS	Volatile solids, in grams per liter
TAN	Total Ammoniacal Nitrogen, in grams per liter
C	Carbon
N	Nitrogen
C/N	Carbon/nitrogen ratio

In this sense, another waste that has recently demanded attention is poultry manure, whose generation has increased due to the growing demand for poultry meat and, as a consequence, the increase in poultry farms and industries. This waste basically consists of a combination of poultry faeces, feathers, non-digestible portion of feed, micro-organisms from the intestinal biota, poultry feed remains, egg shells, etc. Due to their composition, these wastes are highly polluting and require treatment (Gomes et al., 2020; Johannesson et al., 2020; Sillero et al., 2022b). Poultry manure is rich in nitrogen, but also contains significant amounts of phosphorus and potassium. It is very suitable for use as a fertilizer to improve soil properties and fertility due to its composition and nutrient content (Dróżdż et al., 2020).

It is therefore necessary to find a sustainable solution for the treatment of this waste in an environmentally viable way (Sillero and Gustavo, 2022). Anaerobic digestion would meet the objectives of a circular bioeconomy model (Sganzerla et al., 2021), where the reduction of organic waste is achieved, valorising it and obtaining bioenergy and biofertilizers locally (Jurgutis et al., 2020). However, further research is needed for the treatment of organic waste by means of anaerobic digestion variables to improve the performance of the conventional process. Anaerobic co-digestion consists of the joint anaerobic treatment of several wastes taking advantage of the complementarity in composition. This represents an operational improvement as it allows cushioning the temporary variations in composition and production of each waste separately, as well as sharing treatment facilities, unifying management methodologies and reducing investment and operating costs. In addition, the digestion process will achieve greater stability compared to single-stage anaerobic digestión (Montañés Alonso et al., 2016). Another objective that can be achieved with anaerobic co-digestion would be the achievement of a balanced C/N ratio, which would require complementary anaerobic fermentation characteristics between the co-substrates (Pan et al., 2022). Increased biogas production in anaerobic co-digestion processes has been demonstrated in numerous studies with mixtures of different wastes such as sewage sludge and food waste (Li et al., 2018), o pig and poultry manure with the organic fraction of municipal solid waste (Borowski et al., 2014).

It has also been shown that vinasse and poultry manure are an ideal substrate to be combined with sewage sludge due to the good synergy between them, thanks to their high content of dissolved organic matter. It has been shown that poultry manure reduces the risk of acidification of the mixture during the anaerobic digestion of this substrate, increasing the specific methane production by 55% compared to the monodigestion of this waste, creating a very positive synergy in the overall calculation. At the same time, the high ammoniacal nitrogen content of poultry manure can be diluted by the vinasse, avoiding ammonia inhibition (Vanotti et al., 2009; Sillero et al., 2023).

On the other hand, the operating temperature has a strong influence on the process. Thus, microorganisms can operate in mesophilic (35 °C), thermophilic (55 °C) and hyperthermophilic (70 °C) ranges. Temperature accelerates the reactions of the anaerobic digestion process. The temperature phased process consists of carrying out the anaerobic digestion in different thermophilic and mesophilic reactors placed in series. The aim of the TPAcD (Temperature - Phased Anaerobic co-Digestion) technique is to combine the advantages of both processes by reducing their individual limitations and improving the methane production yield, as well as the stability of the process and the quality of the effluent in terms of agronomic properties and absence of pathogens (Riau et al., 2010). With this technology, biohydrogen, biomethane and biofertilizer would be achieved as three final products with high added value.

In order to determine the biodegradation potential of substrates, biochemical hydrogen or methane potential tests (BHP and BMP) are usually developed. These tests consist of mixing the substrates with an inoculum in an anaerobic environment and incubating them at constant temperature and shaking them. These tests are very useful to measure the hydrogen or methane production potential of a substrate or mixture of substrates. The BHP corresponds to the maximum hydrogen production in infinite dark fermentation time and is a key parameter to evaluate the suitability of substrates for biohydrogen production (Tena et al., 2019a). The main conditions for successful hydrogen production are based on pH and temperature. The determination of pH values around 5.5 to favour hydrogen production has been verified by many authors (Chen et al., 2002; Lee et al., 2010; Yang and Wang, 2019). However, the optimal temperature range is not clearly defined, as it depends to a large extent on the characteristics of the raw materials and the inoculum used. The mixture of substrates to be tested for biodegradability is incubated together with the inoculum in batch reactors, the air in the reactor head is purged with the help of nitrogen to achieve anaerobic conditions and the desired operating conditions are programmed. The test is terminated when the gas production is exhausted (Zhang et al., 2016; Alsamet et al., 2019; Tena et al., 2019b).

The aim of this trial is to determine the appropriate temperature range in the anaerobic co-digestion of sludge, vinasse and anaerobic co-digestion of sludge, vinasse and poultry manure for biohydrogen production by means of BHP tests. Subsequently, the effect of temperature and the addition of poultry manure in the first dark fermentation phase on the subsequent biomethane production will be tested. This parameter is evaluated by means of a BMP test, using the acid effluents from the BHP tests. Moreover, two kinetic models were proposed as tools to describe the production of methane produced in the studied scenarios. In this way, the optimal conditions of substrate mixture and temperature would be determined for the design of a TPAcD system to obtain the maximum performance in terms of sustainable green energy generation and organic waste valorisation within the framework of the circular economy. In addition, the results of this study will help to choose the most suitable operating conditions when implementing the system on a larger scale.

2. Materials and methods

2.1. Characterisation of substrates and inoculums

Table 1 shows the average results of the initial physicochemical characterisation with the corresponding standard deviation of the waste used. Both sewage sludge (SS) and vinasse (V) have acidic pH, with vinasse having a more pronounced pH. Poultry manure (PM), on the other hand, shows a slightly basic pH. In all wastes, TCOD has higher values than SCOD, especially in the case of sludge indicating a high proportion of suspended organic matter. Sludge and vinasse have very similar TCOD values. However, poultry manure has a very high TCOD compared to the other wastes. Similarly, the concentration of ST and SV

Table 1
Characterisation of feedstock and initial mixtures.

Parameters	SS	V	PM	SSV	SSVPM
pH	6,47 ± 0,02	3,19 ± 0,01	7,82 ± 0,01	7,20 ± 0,01	7,21 ± 0,00
TCOD (g/L)	49,39 ± 0,01	42,45 ± 0.00	347,96 ± 0.01	47,38 ± 0.00	49,76 ± 0,01
SCOD (g/L)	11,41 ± 0.02	42,22 ± 0.00	238,84 ± 0.01	22,26 ± 0,01	23,91 ± 0,01
TS (g/L)	48,30 ± 0,26	20,20 ± 0,40	600,29 ± 12,17	39,10 ± 0,93	50,53 ± 0,83
VS (g/L)	40,08 ± 0,27	17,50 ± 0,51	423,11 ± 35,66	32,29 ± 1,19	38,88 ± 0,77
Acetic Ac. (mg/L)	546 ± 21	1195 ± 17	25,09 ± 0,74	908 ± 43	1016 ± 47
Propionic Ac. (mg/L)	399 ± 28	47,10 ± 0,4	2,79 ± 0,3	254 ± 16	243 ± 26
Butyric Ac. (mg/L)	83,80 ± 0,75	37,80 ± 5,45	0 ± 0,00	86,46 ± 1,96	81,75 ± 3,26

of poultry manure is very high compared to the other wastes. On the other hand, vinasse and poultry manure show a high proportion of dissolved organic matter. Regarding solids, poultry manure shows the highest ST and SV values, followed by sludge and finally vinasse.

With regard to the mixtures of sewage sludge and vinasse (50:50) (SSV) and sewage sludge, vinasse and poultry manure (49.5:49.5:1) (SSVPM) a pH value slightly above 7 was reached, which was suitable for anaerobic co-digestion. This ratio of substrates was determined in previous trials (Sillero et al., 2021, 2022c). The COD was similar in both mixtures, being slightly higher for the mixture containing poultry manure. As for the solids, the mixture containing poultry manure presents higher values in its concentration, as can be seen in Table 1.

Table 1 shows the three main acids (acetic, propionic and butyric) present in the substrates sewage sludge, vinasse and poultry manure. The residue that stands out most in relation to acetic acid was that of the vinasse, due to the process of generation of this substrate, and which was evidenced by its low initial pH. The sewage sludge has moderate acidity values and, especially in poultry manure, it was practically imperceptible for the three acids. Propionic acid was higher in concentration in sewage sludge than in vinasse and poultry manure. In reference to the mixtures, the concentration of these three acids was quite similar in both mixtures, with slightly higher acetic acid values for the SSVPM mixture.

As for the inoculums, mesophilic, thermophilic and hyperthermophilic sewage sludge inoculums were used, depending on each batch in the BHP trials. The main characteristics of each of them were shown in Table 2.

The parameters presented by the inocula were very similar, with pH above 7, and high COD and solids concentration.

For the subsequent BMP trials, mesophilic sewage sludge inoculum was used for all designed batches.

2.2. Experimental design of BHP and BMP

For the biodegradability tests, batch reactors of 250 ml capacity were used. Each contained 60 ml of substrate mixture + 60 ml of the corresponding inoculum. The pH was adjusted to between 5 and 5.5 for the BHP test by the addition of NaOH or HCl as required for regulation.

The mixing ratios were sewage sludge and vinasse in a 50:50 ratio, and sewage sludge, vinasse and poultry manure in a 49.5:49.5:1 ratio, respectively. These ratios were defined thanks to previous trials carried out in the research group (Sillero et al., 2021, 2022c). Table 3 shows the volume of each substrate added to each reactor.

Batches of each sample were prepared and incubated at different temperature ranges (mesophilic, thermophilic and hyperthermophilic). To each of these mixtures, the inoculum corresponding to its operating temperature range was added in a 50:50 ratio (mixture:inoculum).

A 130 mL headspace was required for gas accumulation and, prior to sealing the bottles, they must be purged with nitrogen to remove any atmospheric gas. The biogas generated, H_2 in the case of the BHP trials, was monitored daily. A physicochemical characterisation of the substrate mixtures used at the beginning and at the end of the test was also carried out to determine the purification and biogas generation performances.

The BMP test was sequential to the BHP and uses the digestate from the previous BHP test to carry out the biodegradability test, adding the new mesophilic inoculum to each of the mixtures to proceed to its initial physicochemical characterisation using the same parameters as in the BHP test.

It is operated in the same way as in the BHP test, using 250 ml batch reactors with a content of 60 ml of mixture + 60 ml of mesophilic inoculum and carrying out the corresponding purge with nitrogen gas. In this case, make sure that the pH of the mixtures is around 7,5 before assembling the bottles to favour the growth of the methanogenic archaea (Montañés et al., 2014; Postawa et al., 2020). NaOH (10M) was used to raise the pH to the desired values. The temperature in this case was set at mesophilic range for all three trials. Biogas production and composition were measured daily and physico-chemical characterisations were performed at the beginning and end of the BMP. Both the BHP and the BMP shall be terminated if, for 3 consecutive days, they produce less than 1% of H_2 or CH_4 of the cumulative total, respectively (Holliger et al., 2016).

2.3. Analytical methods

An initial characterization of the substrates and the feed was carried out, in terms of pH, total solids (TS), volatile solids (VS), total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), volatile fatty acids (VFA) and carbon/nitrogen ratio (C/N ratio).

For TS, VS, TCOD and SCOD parameters, the Standard Methods APHA-AWWA-WPFC [34] were followed.

For the characterization of the pH, a HACH sensION + pHmeter was used. The individual VFAs were determined by gas chromatography, using a gas chromatograph (Shimadzu GC-2010) equipped with a flame ionization detector (FID) system and a capillary column packed with Nukol [34,35]. Acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic and heptanoic acids were quantified in mg/L and the total content of TVFA acids expressed as mgAcHequivalent/L was calculated. For the characterization of both total organic carbon and total nitrogen, a total organic carbon analyzer (Shimadzu TOC-L CSH/CSN) was used, according to 186 the standard APHA-AWWA-WPFC methods [34].

A C9507IS Pressure Meter was used to determine the pressure of the gas contained in the batch reactors. The composition of the biogas was analysed with a gas chromatograph (Shimadzu GC-2010) equipped with a termal conductivity detector (TCD). The column used to determine the composition of H_2, CO_2 and CH_4 was a Supelco Carboxen 1010 plot column [8].

Table 2
Characterisation of the inoculums used for the BHP assays.

Parameters	Mesophilic (M)	Thermophilic (T)	Hyperthermophilic (H)
pH	7,98 ± 0,06	7,76 ± 0,08	7,34 ± 0,10
TCOD (g/L)	37,88 ± 2,02	34,16 ± 2,53	33,97 ± 2,24
SCOD (g/L)	22,91 ± 1,34	20,38 ± 2,14	19,73 ± 1,88
TS (g/L)	30,17 ± 1,55	28,55 ± 1,33	27,92 ± 2,25
VS (g/L)	24,41 ± 1,60	23,12 ± 1,98	21,66 ± 0,97

Table 3
Experimental setup used in this study.

SAMPLES	INOCULUM (mL)	SS (mL)	V(mL)	PM (g)
SSV	60	30	30	0
SSVPM	60	29.7	29.7	10

2.4. Kinetic analysis

For the kinetic analysis of the results, the first-order kinetic model and modified Gompertz model (Scarcelli et al., 2020) were selected. The modified Gompertz model is generally the most widely used model to describe the anaerobic digestion process (Grosser, 2018; Pan et al., 2019; Potdukhe et al., 2021; Ripoll et al., 2020; Zhang et al., 2014). This model relates biogas production to microbial activity and assumes that biogas production follows an exponential increase to reach the maximum level (Ripoll et al., 2020). The kinetic analysis of the methane generation during the BMP was carried out based on the experimental value adjusting the modified equation of the Gompertz kinetic model. The mathematical expressions of the first order kinetic models (Equation (1)) and modified Gompertz (Equation (2)) were shown below:

$$H = P\left[1 - \exp\left(k_{hyd} t\right)\right] \quad (1)$$

$$H = Pexp\left\{ - exp\left[\frac{Rm}{P}(\lambda - t) + 1\right]\right\} \quad (2)$$

where, H = Methane generation (cumulative) in time (t) (ml/gVS), P = potential for maximum methane generation (ml/gVS), Rm = methane generation rate (ml of biogas/gVS/d) and λ = the lag phase during methane generation (d) and t = the time required for the cumulative production of methane (H).

In addition, root mean square error (RMSE) were calculated using Equation (3):

$$RMSE = \left(\frac{1}{n}\sum_{j=1}^{n}\left(\frac{dj}{P}\right)^2\right)^{\frac{1}{2}} \quad (3)$$

where n = number of data; j = jth values; P = measured methane production (mL); and d = deviations between experimental and predicted methane production.

3. Results and discussion

3.1. Evolution of COD and solids

The initial mixtures of each of the tests designed for the development of the BHP tests were analysed, and subsequently the final effluents of each test were analysed, once the production of hydrogen in the reactors had been exhausted.

It can be observed that the pH values varied slightly throughout the trial, remaining at values around 5 and 5.5 throughout the process.

The TCOD values decreased slightly at the end of all trials with respect to the initial values.

With regard to SCOD, it can be observed that in some cases the concentration increases at the end of the test. This result indicates that the organic matter has been solubilised during fermentation, thus increasing its final value with respect to the initial value (Silva et al., 2017). Most of the organic matter was in solid form, but the microorganisms prefer to consume and transform the soluble organic matter. This process of solubilisation of the organic matter was important to make it more available to the microorganisms involved in the process (Chen et al., 2022). As for total and volatile solids, they decreased at the end of all the trials, the difference being greater for the trials carried out in the mesophilic range, indicating that substrate utilisation was higher under mesophilic temperature conditions. Finally, the C/N ratio followed the same trend and decreased at the end of the trials, indicating a greater consumption of carbon by the microorganisms.

3.2. VFA evolution

The addition of the inoculum to the starting mixtures conditioned the VFA concentration at the beginning of the trial. Both mixtures started with an initial concentration of total VFA of around 1300 mg/L. It can be seen in Table 4, how the values of the individual acids studied increase in concentration as the operating temperature increases. This was due to the fact that the thermophilic and hyperthermophilic inocula initially contained a higher contribution of VFA. In dark fermentation of sewage sludge and vinasse (SSV); sewage sludge, vinasse and poultry manure (SSVPM), an increase of all VFA can be seen at the end of all BHP tests (Table 4). During hydrolysis and acidogenesis these VFA were produced, which were important for the subsequent production of hydrogen and methane (Nualsri et al., 2016). The VFA concentrations created at the end of the BHP tests predict the different hydrogen and methane yields that will be produced in the subsequent BMP tests, as the production of the different acids influences the generation of hydrogen and methane (Nualsri et al., 2016). During dark fermentation, acetic and butyric acids were the main metabolites involved in biohydrogen production (Arun et al., 2022). Butyric acid production was directly related to hydrogen production (Kim et al., 2006), This fact was related to the thermophilic assay which, with respect to the initial input, was the one that produced the highest amount of butyric acid, multiplying its concentration at the end of the assay by 10. In the mesophilic assays there were also considerable increases in the amount of final butyric acid, but there were also high concentrations of propionic acid which could have inhibited the production of hydrogen (Han et al., 2020). In the hyperthermophilic test, the concentration of butyric and propionic acids did not change, only the concentration of acetic acid increased.

Table 4
Initial (I) and final (F) characterisation of the mixtures used in the BHP tests for three different temperature ranges.

Parameters		Mesophilic (M)		Thermophilic (M)		Hyperthermophilic (H)	
		SSV	SSVPM	SSV	SSVPM	SSV	SSVPM
pH	I	5,5 ± 0,10	5,45 ± 0,07	5,20 ± 0,09	5,41 ± 0,06	5,29 ± 0,01	5,34 ± 0,00
	F	5,14 ± 0,04	5,07 ± 0,03	5,28 ± 0,05	5,56 ± 0,06	5,20 ± 0,01	5,30 ± 0,01
TCOD(g/L)	I	52,58 ± 2,31	46,47 ± 1,14	49,16 ± 3,03	50,86 ± 3,31	50,83 ± 0,10	48,88 ± 1,39
	F	46,29 ± 1,94	41,63 ± 1,37	38,49 ± 1,89	41,46 ± 1,28	48,81 ± 2,84	46,83 ± 1,90
SCOD (g/L)	I	28,57 ± 1,44	26,76 ± 1,64	32,21 ± 0,99	33,08 ± 1,27	34,59 ± 0,74	30,23 ± 1,53
	F	28,04 ± 1,37	31,70 ± 1,71	27,14 ± 1,78	27,77 ± 0,79	41,63 ± 1,19	44,54 ± 0,94
TS (g/L)	I	41,39 ± 1,75	43,46 ± 2,31	32,16 ± 1,41	35,14 ± 1,22	41,27 ± 0,18	41,03 ± 0,73
	F	32,54 ± 1,47	34,11 ± 1,82	31,48 ± 1,37	33,15 ± 1,41	40,37 ± 0,20	32,80 ± 0,19
VS (g/L)	I	33,06 ± 1,08	34,13 ± 1,58	25,21 ± 1,66	27,27 ± 1,53	31,03 ± 0,17	32,75 ± 0,80
	F	23,63 ± 1,16	26,32 ± 0,84	24,88 ± 1,39	26,26 ± 1,12	29,86 ± 0,53	26,53 ± 0,31
C/N	I	43,12 ± 0,93	28,32 ± 1,01	44,07 ± 2,10	29,85 ± 0,90	45,78 ± 1,22	30,91 ± 0,95
	F	23,42 ± 0,83	18,01 ± 0,73	24,31 ± 1,14	14,66 ± 0,67	25,87 ± 1,01	17,65 ± 0,57
TAN (g/L)	I	1,61 ± 0,02	1,99 ± 0,01	1,62 ± 0,05	1,92 ± 0,04	1,71 ± 0,03	2,03 ± 0,03
	F	6,92 ± 0,13	7,33 ± 0,011	4,23 ± 0,08	4,89 ± 0,07	7,91 ± 0,12	8,16 ± 0,09
Acetic Ac.	I	714 ± 55	432 ± 16	1187 ± 59	1116 ± 958	1803 ± 122	1468 ± 35
	F	2555 ± 134	2598 ± 153	1934 ± 113	1899 ± 157	2299 ± 138	1828 ± 105
Propionic Ac.	I	289 ± 27	276 ± 24	713 ± 64	686 ± 24	595 ± 22	610 ± 19
	F	1302 ± 64	1544 ± 85	756 ± 46	1274 ± 31	710 ± 46	712 ± 42
Butyric Ac.	I	101 ± 12	143 ± 11	134 ± 20	122 ± 9	1347 ± 39	1620 ± 53
	F	978 ± 58	1066 ± 37	1307 ± 93	1298 ± 100	1639 ± 102	1801 ± 109

The ratio between the concentration of butyric acid and acetic acid was important to determine the effectiveness of hydrogen production. Several authors establish an optimal range of butyric acid/acetic acid between 0,4 and 2,1 (Zahedi et al., 2013; Angeriz-Campoy et al., 2015; Mahmoodi et al., 2022). For the six BHP tests developed, the calculation of this ratio yielded the following values (0.38 and 0.41 for SSV and SSVPM, respectively, in the mesophilic range, 0.68 for both mixtures in the thermophilic range, and 0.71 and 0.98 for SSV and SSVPM, respectively, in the thermophilic range. All values were within the established range except for the SSV mixture in the mesophilic range with a value of 0.38. However, the mesophilic and hyperthermophilic range tests suffered from inhibition of hydrogen production, as will be seen in the next section. Thus, the initial total VFA concentration was higher in the hyperthermophilic range tests, due to a higher input from the hyperthermophilic inoculum. This resulted in a higher VFA concentration at the end of the hyperthermophilic test, which would have consequences for methane production in the BMP tests designed afterwards (Tena et al., 2021b).

3.3. Evolution of TAN

Hydrogen production was highly dependent on pH, temperature and ammonia concentration. In the process of hydrogen production by dark fermentation at acidic pH, ammonia was mainly in the form of NH_4^+. High concentrations of this compound do not affect the concentration of H_2 in the gas produced, but they do affect the total gas production rate, reducing it. At mesophilic and hyperthermophilic temperatures, an ammonia concentration >200 mg/L would lead to inhibition by decreased microbial activity, which would result in a lower conversion of substrate to hydrogen (Chen et al., 2008). For mesophilic BHP tests, total ammonia nitrogen (TAN) concentrations of up to 7 g/L were recorded, and in the hyperthermophilic range, TAN values above 8 g/L were reached. A high TAN concentration in these circumstances and at pH around 5.5, would inhibit the activity of hydrogen producers due to interference with the intracellular pH and therefore an increase in the maintenance energy requirement of the microbial cells and inhibition of the activities of the enzymes responsible for the Hydrogen (Chen et al., 2021). For the BHP tests carried out in the thermophilic range, TAN values between 4 and 5 g/L were recorded, much lower than for the rest of the samples, which may explain the higher hydrogen production recorded. This would indicate that the limit of inhibition of hydrogen production for the mixture of sewge sludge and vinasse; sewage sludge, vinasse and poultry manure would be between 5 and 7 g/L.

3.4. Hydrogen production and hydrogen potential

Fig. 1. Shows the hydrogen production for each tested temperature range of the sewage sludge and vinasse mixtures (SSV) and the sewage sludge, vinasse and poultry manure mixtures (SSVPM).

It can be observed that the test carried out in the thermophilic temperature range was the most beneficial for hydrogen production, while the tests carried out in the mesophilic and thermophilic range registered very low hydrogen production values, showing that these temperatures do not favour hydrogen release. In turn, the mixture containing poultry manure achieved higher hydrogen production and yield values, reaching its maximum yield at 27.1mLH_2/gVS in the thermophilic range. This may have been due to a balanced C/N ratio achieved by the addition of poultry manure, thus favouring the activity of the microorganisms (Kainthola et al., 2019). In the literature we can find BHP tests with different substrates that obtained similar yields, such as BHP carried out with food waste where a yield of 39.14mLH_2/g food waste was obtained (Han et al., 2015) or with activated sludge, resulting in a yield of 11,01mLH_2/gVS (Li et al., 2022) also carried out in thermophilic temperature ranges.

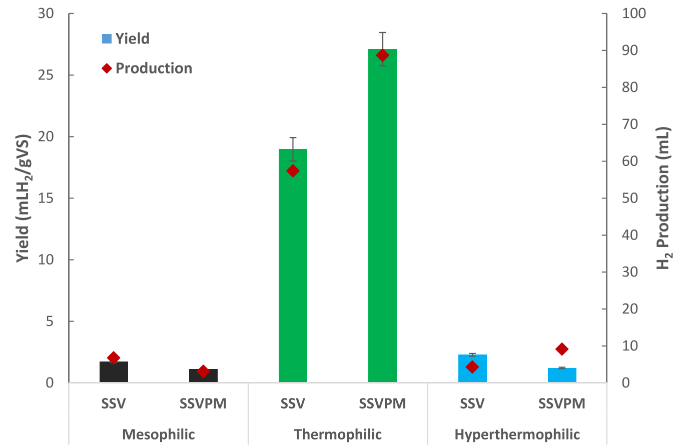

Fig. 1. Accumulated hydrogen production in mL during the BHP tests, of the SSV and SSVPM mixtures, in each temperature range considered and hydrogen yield for each BHP in the different temperature ranges considered expressed as mLH_2/gVS.

3.5. Evolution of the BMP tests

The main parameters measured at the beginning and at the end of the mesophilic BMP tests, carried out with the acid effluent of the mesophilic, thermophilic and hyperthermophilic BHP tests on the sewage sludge:vinasse (SSV) and sewage sludge:vinasse:poultry manure (SSVPM) mixtures, were shown below (Table 5).

It can be observed how the pH increased in all the tests carried out until reaching values close to 8. With regard to COD, a decrease was observed at the end of all the tests, being this decrease more important for the samples coming from the BHP test in the hyperthermophilic range. As for total and volatile solids, the trend was similar, showing a decrease at the end of all tests, the percentages of elimination of these parameters will be treated in detail in the following section.

With regard to the VFA, a drastic consumption was observed, which translates into a significant decrease in the effluent resulting from all the tests. Acetic acid was reduced at the end of the tests to values below 400 mg/L, being the tests coming from the BHP in the hyperthermophilic range the ones that suffer a greater decrease in the final effluent, with values below 200 mg/L. Propionic acid disappears completely in the effluent of all tests. And butyric acid is reduced to values below 250 mg/L in the effluents, being undetectable in the BMP effluents coming from the BHP in thermophilic range.

Finally, the C/N ratio decreased in all the cases studied, indicating that slightly more carbon was consumed in relation to the available nitrogen.

3.6. Removal efficiencies

It can be observed in Fig. 2 that the highest percentages of TCOD (69.30%), SCOD (79.51%), TS (34.00%) and VS (44.00%) removal were recorded for BHP SSVPM in thermophilic range. For the tests that were carried out first in the hyperthermophilic range, slightly lower values were recorded than those mentioned above, being slightly higher for the SSV mixture. The lowest depuration efficiencies were recorded for SV and SSVPM which performed in the mesophilic range in BHP and BMP. Similar values were recorded by Cabbai et al. (2013) in BMP trials with food waste, where they reported SV removal values of around 33% and SCOD removal values of around 69% (Cabbai et al., 2013).

3.7. Methane production and yields

For the experiments carried out with the thermophilic and hyperthermophilic effluents of the BHP, it can be observed in Fig. 3. The

Table 5
Characterisation of the effluents resulting from the reinoculated BHP tests (I), and characterisation at the end (F) of the BMP tests developed in mesophilic range.

Parameters		Mesophilic (M)		Thermophilic (T)		Hyperthermophilic (H)	
		SSV	SSVPM	SSV	SSVMP	SSV	SSVPM
pH	I	7,21 ± 0,01	7,20 ± 0,02	7,19 ± 0,03	7,67 ± 0,04	7,02 ± 0,01	7,13 ± 0,01
	F	8,06 ± 0,02	8,05 ± 0,03	7,92 ± 0,06	8,06 ± 0,07	8,39 ± 0,01	8,23 ± 0,02
TCOD (g/L)	I	48,88 ± 1,02	53,64 ± 3,18	37,96 ± 1,25	45,07 ± 1,38	49,51 ± 0,78	49,68 ± 0,22
	F	33,70 ± 0,99	35,14 ± 1,94	22,64 ± 1,10	16,27 ± 1,54	18,03 ± 2,50	16,48 ± 1,32
SCOD (g/L)	I	25,53 ± 0,77	26,79 ± 1,13	25,07 ± 0,96	27,64 ± 1,87	39,41 ± 3,72	27,42 ± 0,40
	F	16,26 ± 1,33	16,20 ± 0,84	13,20 ± 0,77	5,67 ± 0,54	10,05 ± 1,75	7,91 ± 0,72
TS (g/L)	I	37,03 ± 1,24	38,61 ± 1,22	29,52 ± 0,94	35,04 ± 3,14	40,98 ± 0,55	39,16 ± 1,60
	F	29,76 ± 0,50	31,34 ± 0,67	22,40 ± 0,80	23,21 ± 1,18	27,92 ± 0,09	30,56 ± 7,02
VS (g/L)	I	25,81 ± 0,62	28,05 ± 2,01	22,06 ± 1,03	26,33 ± 1,39	30,41 ± 0,07	30,36 ± 0,34
	F	19,61 ± 0,72	20,96 ± 1,24	15,15 ± 0,64	15,41 ± 1,28	17,46 ± 0,13	22,09 ± 6,89
Acetic Ac. (mg/L)	I	1438 ± 111	1399 ± 124	1184 ± 72	1046 ± 126	1780,00 ± 46,23	949,03 ± 26,33
	F	373 ± 29	354 ± 18	292 ± 12	355 ± 12	191,57 ± 5,11	163,20 ± 5,81
Propionic Ac. (mg/L)	I	687 ± 32	711 ± 47	537 ± 28	623 ± 21	580,32 ± 16,51	323,01 ± 11,98
	F	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0,00 ± 0,00	0,00 ± 0,00
Butyric Ac. (mg/L)	I	594 ± 27	312 ± 14	651 ± 18	706 ± 27	1271,46 ± 33,60	813,01 ± 21,98
	F	121 ± 9	234 ± 11	0 ± 0	0 ± 0	50,84 ± 5,93	112,85 ± 6,23
C/N	I	25,18 ± 0,22	22,01 ± 0,19	24,24 ± 0,25	24,81 ± 0,17	26,83 ± 0,14	23,93 ± 0,17
	F	21,42 ± 0,30	17,14 ± 0,14	13,38 ± 0,13	14,02 ± 0,11	20,62 ± 0,12	17,70 ± 0,16

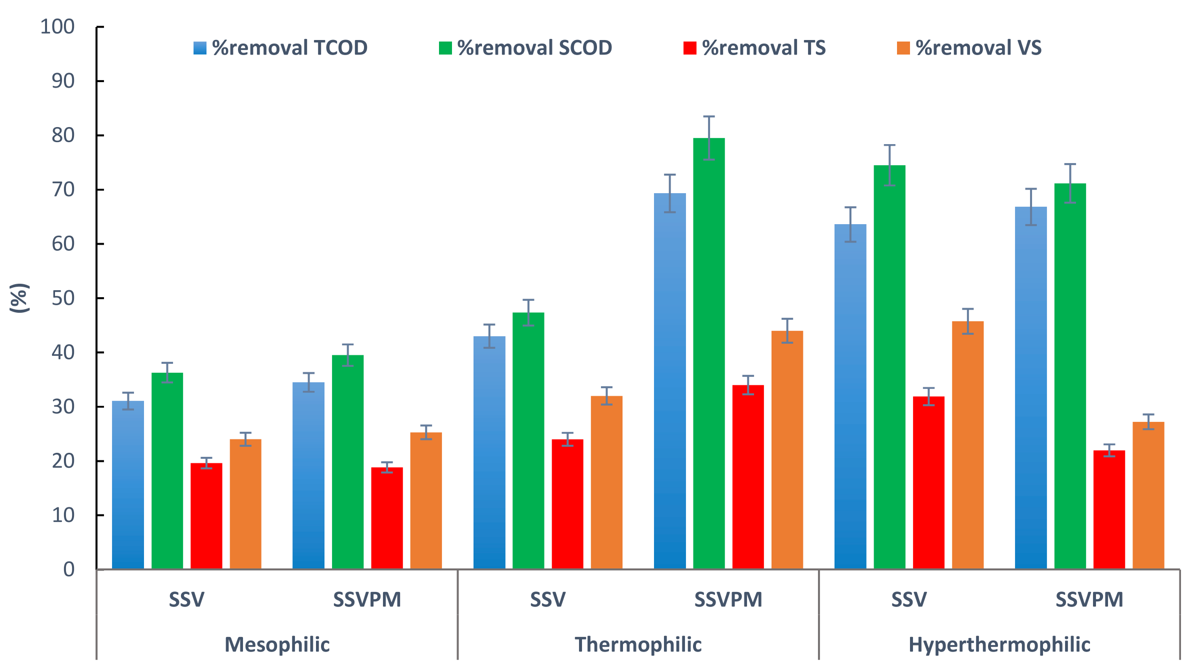

Fig. 2. TCOD, SCOD, TS and VS removal efficiencies at the end of the BMP test in mesophilic range, with effluents from the previous BHP test at different temperature ranges.

thermophilic effluent started more smoothly, stabilising much earlier than the hyperthermophilic effluent, which continued to produce methane for both mixtures until reaching values above 400 mL accumulated, being higher for the sludge and vinasse mixture (428 mL) than for the mixture containing poultry manure (413 mL), which was slightly lower. For the trial with mesophilic effluent from the BHP, methane production in the first days occurred more abruptly than in the other trials, but the curve stabilised around 8 days of the trial, with very similar maximum values for the two mixtures, around 199 mL for the mixture with poultry manure and 207 mL for the mixture of sludge and vinasse alone.

It should be noted that only in the test with thermophilic effluents, the mixture containing poultry manure recorded a higher value of accumulated methane (162 vs. 136 mL of CH_4). For the rest of the trials, the mixture of sludge and vinasse alone was the one with the highest cumulative CH_4 values.

Regarding methane production and yield, it is clearly observed in the figure, that the trials that were developed first in hyperthermophilic range, obtained a higher methane yield that exceeded 50% with respect to the other trials coming from BHP in mesophilic and thermophilic ranges. Therefore, hyperthermophilic temperatures in a first stage would not be suitable for hydrogen production, but would favour methane production later on. Several researchers have reported sequential hydrogen and methane production studies with different substrates, such as the BHP and BMP assays performed by Jariyaboon et al. (2015), using skimmed latex serum as substrate in thermophilic temperature range for both assays where yields of 4LH_2/L-s and 12 LCH_4/L-s, respectively, were obtained (Jariyaboon et al., 2015). 132 nmLCH_4/gVS were recorded in feed waste BMP trials with sludge inoculum from BHP trials (Reilly et al., 2016).

3.8. *Kynetic analysis*

First Order kinetic models and Modified Gompertz were used to

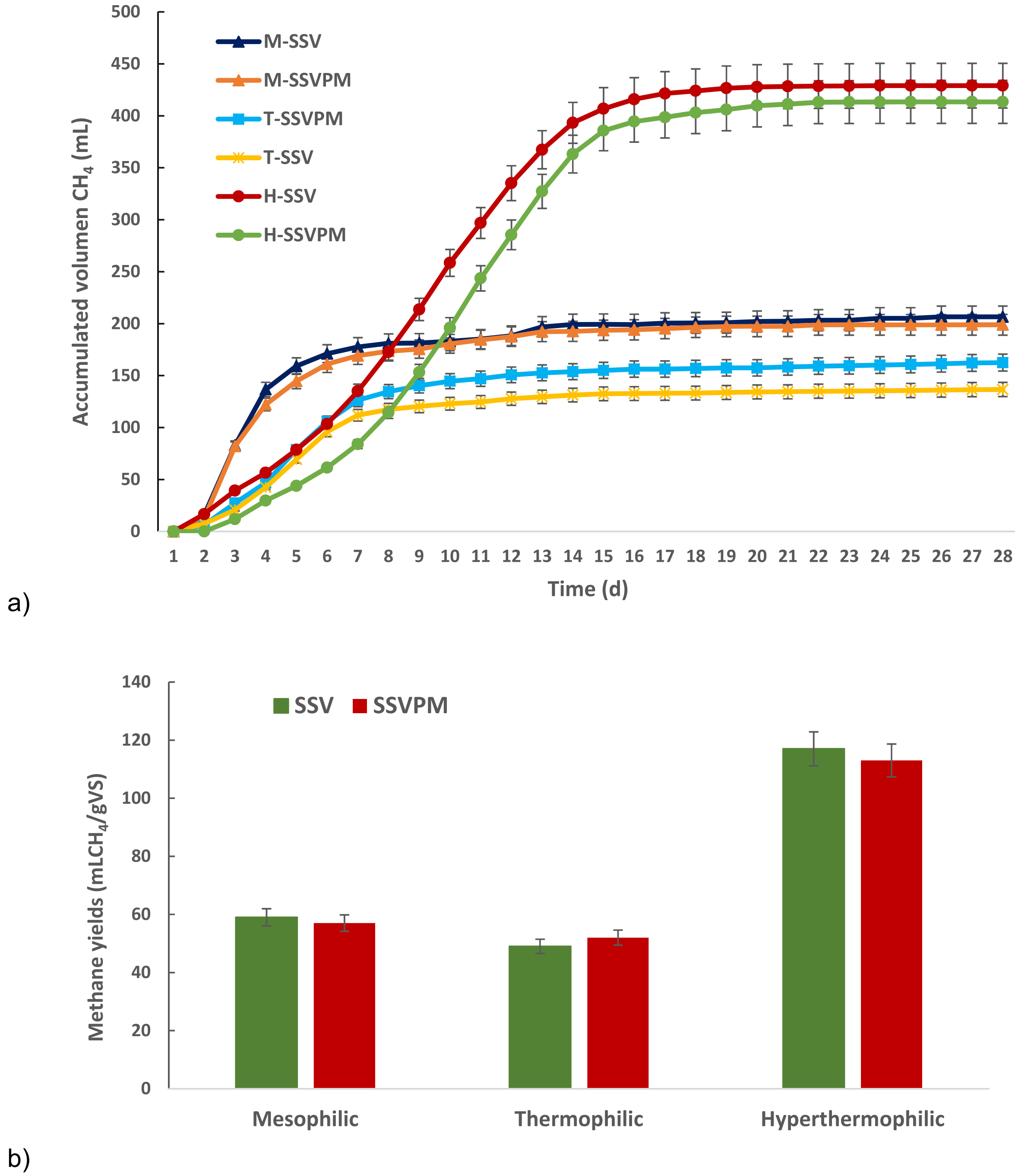

Fig. 3. a) Cumulative methane production for the BMP trials with effluents from BHP Mesophilic (M), thermophilic (T) and hyperthermophilic (H), from sludge and vinasse (SSV) and sludge, vinasse and poultry manure (SSVPM) mixtures. b) Methane yield expressed as mLCH$_4$/gVS for each BMP trial carried out.

predict methane production during the BMP tests developed. Table 6 shows the results obtained in each model for the sewage sludge and vinasse mixtures, and the sewage sludge, vinasse and poultry manure mixtures, from the BHP tests developed in different temperature ranges (mesophilic, thermophilic and hyperthermophilic).

The data obtained from the kinetic analysis showed that the difference between the real experimental data and the estimated data was relatively small, however, in the First Order Model for the samples that developed in the first stage with hyperthermophilic temperatures, much higher percentages were found, showing that this model would not be adequate to predict methane production in this case. As for the R^2 values, the Modified Gompertz Model presents data closer to 1, with values between 0.983 and 0.9984. For the First Order Model, the R^2 values were slightly lower, between 0.9567 and 0.9889, with the lowest values again for the samples in the hyperthermophilic range. Finally, the VER and RMSE values were slightly higher for the First Order Model in the mesophilic and thermophilic range. For the samples in the hyperthermophilic range, these values were 4 times higher. Therefore, it can be stated that the Modified Gompertz Model was a better fit to the experimental data than the First Order Model. Many authors found a better fit to their experimental data using the Modified Gompertz Model (Wang and Wan, 2009; Gaur and Suthar, 2017).

This study helps to clarify the choice of the process and its operating conditions depending on the objective to be achieved. In other words, if the aim is to achieve a higher yield in hydrogen production, thermophilic temperatures should be selected in the first stage, while if the

Table 6
Kinetic analysis for the methane production of SSV and SSVPM with different temperaturas in the acidogenic stage.

MODELS	PARAMETERS	Mesophilic		Thermophilic		Hyperthermophilic	
		SSV	SSVPM	SSV	SSVPM	SSV	SSVPM
FIRST ORDER KINETICS	P(mL)	203.0900	198.5161	139.4092	165.3089	543.8252	634.7118
	Difference(%)	1.3210	0.1751	2.0000	1.7080	21.1125	34.8770
	K_{hid}(1/h)	0.0128	0.0120	0.0083	0.0079	0.0031	0.0020
	R^2	0.9844	0.9889	0.9777	0.9803	0.9696	0.9567
	Adj R^2	0.9678	0.9772	0.9543	0.9595	0.9378	0.9121
	VER (Standard error of estimate)	9.6853	8.0455	8.9313	10.0029	39.3682	48.4299
	RMSE(Root mean square error)	9.3312	7.7528	8.604	9.6380	37.9360	46.67
MODIFIED GOMPERTZ	P(mL)	198.0170	193.7777	135.2292	157.9230	460.4820	423.0700
	Difference(%)	4.2971	2.4960	1.0180	2.8070	6.8340	2.2990
	R_m (mL/h)	12.8667	1.8015	0.7666	1.1703	1.2608	1.9471
	λ(h)	2.1957	8.9370	0.0001	28.4240	0.0002	105.1956
	R^2	0.9891	0.9909	0.9898	0.9984	0.9839	0.9973
	Adj R^2	0.9767	0.9803	0.9781	0.9965	0.9656	0.9942
	VER	8.2447	7.4623	6.1792	2.9219	29.2723	12.4514
	RMSE	7.7896	7.0508	5.8370	2.7608	27.6800	11.7600

desired objective is to boost methane production, the operating conditions in the first stage should be hyperthermophilic. Therefore, when designing such technology on a larger scale, the selected temperature ranges should be taken into account depending on the desired purpose (Ruíz and responsable de, 2021).

4. Conclusions

The BHP trials at different temperatures showed very significant differences in the quantification of hydrogen production. The thermophilic temperature favours hydrogen production in the first stage of dark fermentation reaching yields of 27 mLH_2/gVS for the SSVPM mixture, however, at mesophilic and thermophilic temperatures, H_2 production yields were practically null, possibly due to accumulated TAN values that exceeded the production inhibition limits. On the other hand, in the BMP tests, hyperthermophilic temperatures in the first stage of the process favour the subsequent methane production considerably, reaching yields of 117 and 113 $mLCH_4$/gVS for the SSV and SSVPM mixtures, respectively, compared to the yields between 49 and 59 $mLCH_4$/gVS that were recorded for all mixtures in the mesophilic and thermophilic ranges. The Modified Gompertz model was the best fit to the experimental data, with $R^2 > 0.983$ in all cases. These results were very interesting when designing a larger scale waste valorisation system with temperature phase separation, depending on the desired objective of enhancing hydrogen or methane production, the operating conditions chosen will be different.

CRediT authorship contribution statement

Leonor Sillero: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft. **Rosario Solera:** Conceptualization, Resources, Writing – review & editing, Project administration, Funding acquisition. **Montserrat Perez:** Conceptualization, Resources, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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