Title: Enhanced Hydrogen Production from Sewage Sludge by Cofermentation with Wine Vinasse

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Abstract

The cofermentation of sewage sludge and wine vinasse at different mixing ratios to enhance hydrogen production was investigated. Batch experiments were carried out under thermophilic conditions with thermophilic sludge inoculum obtained from an acidogenic anaerobic reactor. The results showed that the addition of wine vinasse enhances the hydrogen production of sewage sludge fermentation. The highest hydrogen yields, 41.16±3.57 and 43.25±1.52mL H_2/g VS_{added}, were obtained at sludge:vinasse ratios of 50:50 and 25:75, respectively. These yields were 13 and 14 times higher than that obtained in the monofermentation of sludge (3.17±1.28mL H_2/g VS_{added}). The highest VS removal (37%) was obtained at a mixing ratio of 25:75. Cofermentation had a synergistic effect the hydrogen yield obtained at a sludge:vinasse ratio of 50:50 was 40% higher, comparing to the sum of each waste. Furthermore, kinetic analysis showed that Cone and first-order kinetic models fitted hydrogen production better than the modified Gompertz model.

Keywords: Hydrogen production; Cofermentation; Sludge; Wine vinasse

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1. Introduction

The use of fossil fuels has led to increased concentrations of greenhouse gases in the atmosphere. As a result, numerous studies have been carried out to obtain clean energy. Hydrogen is currently considered one of the major energy carriers of the near future because it is a clean combustion product with a high heating value (122kJ/g) [1,2]. Dark fermentation (DF) is used to produce H_2 due to the fact it offers several advantages, such as its high production efficiency, low treatment cost and simplicity of operation [3,4]. DF is a biological process in which bacteria degrade carbohydrates and generate hydrogen together with volatile fatty acids (VFA) and CO_2. This process comprises the first two phases of anaerobic digestion (hydrolysis and acidogenesis). Different kinds of waste have been used for the production of hydrogen, including sewage sludge [5], food waste [6], cassava stillage [7], algal biomass [8] and the organic fraction of municipal solid waste [1]. Among these wastes, sewage sludge has increasingly become the main waste for dark fermentation due to its high organic and nutrient content and the increasing amount generated at wastewater treatment plants (WWTPs). However, sewage sludge fermentation achieves a low hydrogen yield. Therefore, cofermentation of sewage sludge with other waste, such as glycerol [9], food waste [4,10], ryegrass [2] or fallen leaves [11], has been studied to enhance the hydrogen yield.

Besides the aforementioned co-substrates, wine vinasse is a promising option as a cofermentation substrate for sewage sludge. Wine vinasse is the final by-product of wine distillation, with 10-15L being generated for each litre of alcohol produced. This waste has a low pH and high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), so its discharge into the environment before treatment is harmful and has a high pollution potential [12–14]. Wine vinasse shows great potential as a co-substrate for sludge hydrogen fermentation because of its surplus organic load.

Moreover, wine vinasse is a seasonal waste, so cofermentation with sewage sludge has the advantage of being able to share the same treatment facilities.

Biochemical Hydrogen Potential (BHP) tests have been widely applied to evaluate the amount of hydrogen that can be produced when waste is biodegraded under fermentative conditions. pH and temperature are two important parameters in the process due to the fact that the growth of each type of microorganism depends on them [15]. Several studies report that the optimal pH for enhanced hydrogen production ranges between 5 and 6 [1,16,17]. As regards temperature, both thermophilic (55ºC) and mesophilic (35ºC) conditions have been used in BHP tests. Although DF under mesophilic conditions has been widely applied, DF under thermophilic conditions has come increasingly to the fore in recent years, mainly because increasing the temperature has a positive effect both on the thermodynamics and kinetics of the process. Furthermore, high temperatures destroy a greater proportion of pathogenic organisms and reduce hydrogen-consuming bacteria [18,19].

The BHP tests are a first step to verify the improvement in the hydrogen production of the digestion of sewage sludge by adding different percentages of wine vinasse. Subsequently, these studies should be carried out on a larger scale, including a pilot and semi-industrial scale to corroborate the results of hydrogen generation and proceed to its industrial implantation [20].

The cofermentation of sewage sludge and wine vinasse, in addition to producing hydrogen, which means a reduction in the use of fossil fuels, solves the environmental problem posed by the generation of both wastes and their subsequent disposal in the environment.

The suitability of the modified Gompertz model to describe and model a batch fermentative hydrogen production process has been successfully proved [11,21–23]. However, the use of other models, such as the Cone and first-order kinetic models, is still limited. Some studies [2,24,25] have recently used the aforementioned models to compare and analyse different hydrogen production options.

In this study, wine vinasse was added as a co-substrate in sewage sludge fermentation at different mixing ratios with the aim of investigating an effective and practically feasible method to enhance hydrogen production from sewage sludge. Hydrogen production from sewage sludge co-fermented with wine vinasse has rarely been investigated, therefore the need arises to carry out this study. Furthermore, kinetic models were used to analyse the behaviour of hydrogen production.

2. Materials and methods

2.1. Substrates

The substrates used in the batch tests were sewage sludge (SS) from Guadalete municipal wastewater treatment plant in Jerez de la Frontera, Cadiz, Spain, and wine vinasse (WV) from the González Byass winery, also located in Jerez de la Frontera, Cadiz, Spain. The SS and WV samples were stored at 4ºC and -20ºC, respectively, prior to use so as to avoid their degradation at room temperature.

The SS:WV mixing ratios in the feedstock were set at 100:0, 75:25, 50:50, 25:75 and 0:100 based on volume, respectively.

2.2. Inoculum

The inoculum used as seed for the batch tests was obtained from a laboratory scale semi-continuous acidogenic thermophilic anaerobic digester treating waste activated sludge for hydrogen production. The reactor operated at pH 5.5, temperature of 55°C and HRT of 4 days.

2.3. Biochemical hydrogen potential

250mL glass bottles with a 120mL working volume and a 130mL headspace volume were used to assess hydrogen fermentation performance. A mixing ratio of inoculum to feedstock of 1:1 (v/v) [16,22,23] was used for each reactor. The initial pH of each bottle was set at 5.5, a value at which methanogenic *Archaea* are inhibited [16]. All the bottles were purged with nitrogen gas for 5 min to ensure an anaerobic environment and incubated in an orbital shaker under thermophilic conditions (55°C).

All tests were carried out in triplicate and the average values are shown. Three bottles were used as control (only inoculum, without any substrate). The hydrogen production from the control was subtracted from the hydrogen production obtained in the substrate tests prior to data analysis.

2.4. Analytical methods

Inoculum and substrates were characterized in terms of pH, total solids (TS), volatile solids (VS), total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD) and volatile fatty acids (VFA). These determinations are those that are usually performed with the purposes of process monitoring and control of the fermentation bottles.

TS, VS, TCOD and SCOD were analysed according to the Standard Methods [26]. pH was measured using a Crison 20 Basic pH meter [26]. VFA were determined by gas chromatography on a gas chromatograph (Shimadzu GC-2010) equipped with a flame ionization detector (FID) and a capillary column filled with Nukol [27].

Both the volume and composition of the biogas produced in the bottles were measured daily. Volumetric biogas production was quantified indirectly by measuring the pressure inside the bottles. This pressure can be converted to volume according to the ideal law of gases [28]. Gas volumes were converted to standard conditions [29]. The composition of the biogas was determined by gas chromatography separation on a Shimadzu GC-2010 system equipped with a thermal conductivity detector (TCD), using a Supelco Carboxen 1010 Plot column [30]. The analysed gases were H_2, CO_2, CH_4 and O_2.

2.5. Kinetic analysis

The cumulative hydrogen production from sewage sludge and wine vinasse cofermentation was analysed using the modified Gompertz (Eq. (1)), Cone (Eq. (2)) and first-order kinetic (Eq. (3)) models.

$$\mathrm{H{=}P\,exp}\left\{\text{-}\ \mathrm{exp}\left[\frac{\mathrm{R_m e}}{\mathrm{P}}(\lambda-\mathrm{t})+1\right]\right\} \tag{1}$$

$$\mathrm{H}=\frac{\mathrm{P}}{1+\left(\mathrm{k_{hyd}t}\right)^{-\mathrm{n}}} \tag{2}$$

$$\mathrm{H}=\mathrm{P}\left[1-\exp\left(-\mathrm{k_{hyd}t}\right)\right] \tag{3}$$

where H represents the cumulative hydrogen volume (mL), P is the hydrogen production potential (mL), R_m is the maximum hydrogen production rate (mL/h), e is equal to 2.718, λ is the lag phase time (h), t represents the fermentation time (h), k_{hyd} is the hydrolysis rate constant (1/h) and n is the shape factor.

3. Results and discussion

3.1. Characterization of inoculum and substrates

The physico-chemical characteristics of the inoculum and substrates are summarized in Table 1. As can be seen, SS has a higher organic matter content than WV in terms of VS and TCOD. However, WV has a higher content in soluble organic matter, as shown in the SCOD results.

Since WV has a higher concentration of soluble chemical oxygen demand than sewage sludge, their mixture increases the concentration of solubilized organic matter with respect to SS alone. This indicates that the mixture of the two may be considered a promising substrate for hydrogen production.

Parameters	Units	Inoculum	SS	WV
pH		5.39	6.56	3.21
TS	g/L	26.21	38.66	27.40
VS	g/L	19.18	32.50	18.16
TCOD	g/L	51.30	51.14	38.38
SCOD	g/L	31.49	6.33	38.09
Total VFA	g/L	6.05	0.91	1.18
C/N ratio			5.10	25.33

Table 1. Physico-chemical characteristics of the inoculum and substrates

3.2. Characterization of the tests

In Table 2, physical-chemical characteristics are summarized for the different sludge-vinasse mixtures assayed at the beginning and end of experiments. As can be seen in Table 2, the initial pH in each assay was adjusted around 5.5 (5.35-5.41) in order to enhance hydrogen generation [22]. The pH remained stable between 5.2 and 5.7 during the dark fermentation for all mixing assayed. This suggest the buffer capacity of the system, favouring microbial activity [31].

Parameters		pH	TS	VS	TCOD	SCOD	Total VFA
Units			g/L	g/L	g/L	g/L	g/L
100:0	Initial	5.41 ± 0.05	31.65 ± 0.17	25.38 ± 0.46	51.78 ± 1.13	22.14 ± 0.65	2.97 ± 0.59
	Final	5.63 ± 0.04	24.82 ± 0.05	18.49 ± 0.13	47.33 ± 2.56	31.25 ± 1.20	6.23 ± 0.08
75:25	Initial	5.46 ± 0.02	29.95 ± 0.02	23.79 ± 0.06	54.04 ± 1.00	25.03 ± 0.65	3.19 ± 0.19
	Final	5.55 ± 0.03	23.07 ± 0.20	17.47 ± 0.59	46.46 ± 1.64	31.25 ± 1.58	5.97 ± 0.42
50:50	Initial	5.35 ± 0.03	28.67 ± 0.50	22.59 ± 0.46	52.91 ± 0.85	30.98 ± 1.02	3.38 ± 0.24
	Final	5.28 ± 0.04	22.40 ± 0.50	16.39 ± 0.40	46.14 ± 0.89	31.59 ± 0.47	6.23 ± 0.18
25:75	Initial	5.43 ± 0.07	27.30 ± 0.15	20.91 ± 0.19	52.53 ± 5.25	32.22 ± 0.40	3.63 ± 0.12
	Final	5.25 ± 0.06	19.72 ± 0.39	13.16 ± 0.40	41.31 ± 0.71	30.60 ± 0.67	6.59 ± 0.32
0:100	Initial	5.39 ± 0.03	26.73 ± 0.23	19.47 ± 0.46	49.75 ± 2.17	38.92 ± 0.82	3.77 ± 0.45
	Final	5.23 ± 0.05	15.45 ± 1.40	9.89 ± 0.88	40.50 ± 0.42	32.53 ± 0.28	6.85 ± 0.19

Table 2. Physico-chemical characteristics of the fermentation tests.

3.2.1. VS removal

The VS removals obtained in each test are shown in Fig. 1. VS removal is the parameter normally applied to express the efficiency of waste reduction in fermentative hydrogen production. VS removal was 27.13% for monofermentation of sewage sludge. VS removal does not improve at sludge:vinasse ratios of 75:25 and 50:50. However, VS removal was higher (37.07%) at the mixing ratio of 25:75. The highest VS removal, 49.22%, was obtained from wine vinasse monofermentation. VS removal at a mixing ratio of 25:75 was comparable to municipal solid waste and sludge cofermentation (34%) [16] and higher than sludge and perennial ryegrass cofermentation (21.8%) [2] or rice straw and sludge cofermentation (13%) [32].

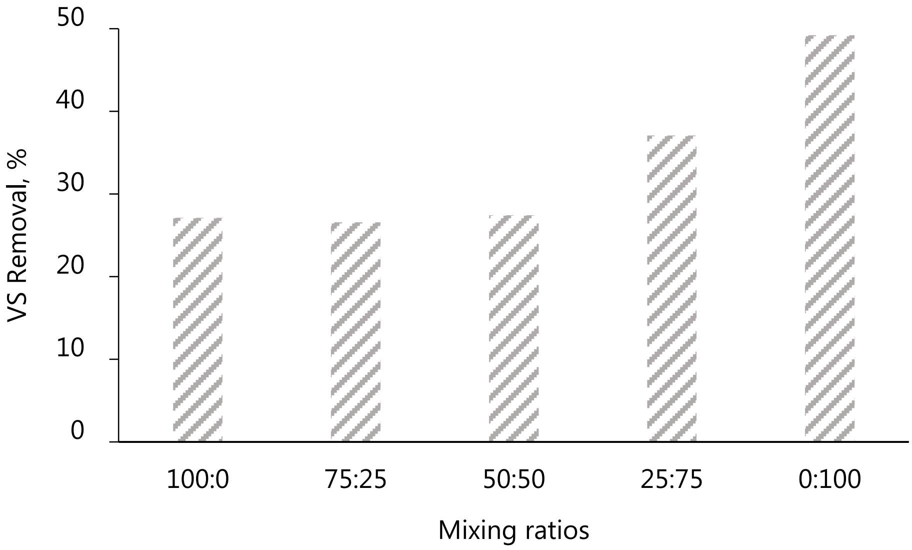

Figure 1. VS removal for cofermentation of sewage sludge with wine vinasse at different mixing ratios (SS:WV).

3.2.2. SCOD evolution

The amount of SCOD represents the concentration of soluble organic matter. SCOD removal is a parameter that expresses the degree of hydrolysis and solubilisation achieved by acidogenic bacteria.

As shown in Fig. 2, the initial SCOD concentration increased with the increase in the proportion of wine vinasse (22.14±0.65 -38.92±0.82g/L). After fermentation, the SCOD concentration increased during both sewage sludge monofermentation and cofermentation with 25% wine vinasse. However, SCOD does not undergo any change at a mixing ratio of 50:50 and decreases for the other mixing ratios, with SCOD removals of 16.42% and 5.03% in wine vinasse monofermentation and cofermentation of sludge with wine vinasse at a mixing ratio of 25:75, respectively. Initially, only hydrolysis of organic compounds occurs. However, the decrease in SCOD due to the consumption of SCOD via acidogenesis was greater than its production. As no data was found in the literature regarding the cofermentation of sludge with wastes of similar characteristics to vinasse, a comparison was made with different types of waste. Lin et al.[33] found that SCOD concentrations increased in the first hours of cofermentation of pulp and paper sludge with food waste and then decreased at the end of hydrogen production. Zhou et al.[34] observed that SCOD concentrations increased during cofermentation of food waste with sludge at various mixing ratios. However, lower SCOD concentrations of 29.8-38.2% were obtained during hydrogen fermentation with enzyme pretreated sludge. Yang and Wang [2] reported that this difference between different studies might be due to the fact that different types and compositions of feedstock and different inoculum sources result in a major variation in hydrolysis efficiency of particular organic materials and the utilization efficiency of soluble organic matter.

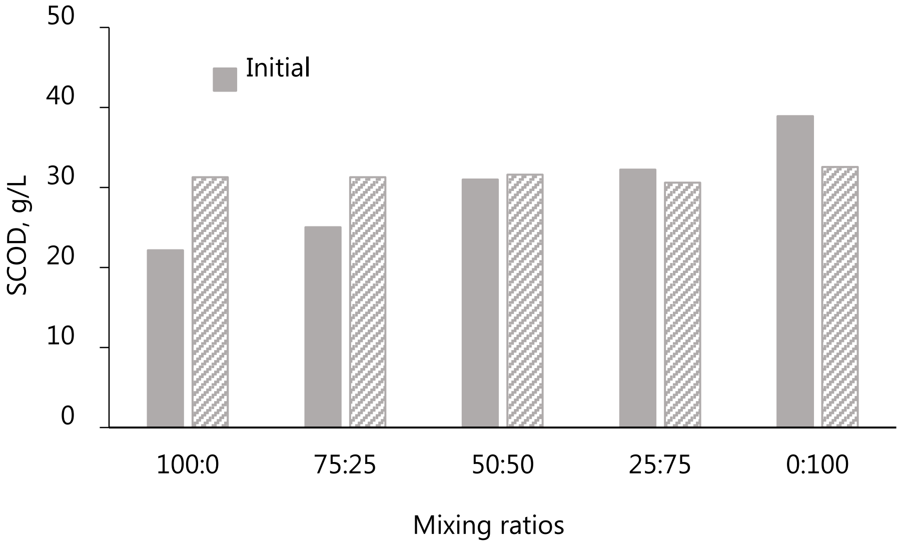

Figure 2. Change in SCOD in the cofermentation of sewage sludge with wine vinasse at different mixing ratios (SS:WV).

3.2.3. VFA production

Hydrogen production is commonly accompanied by the formation of VFA during the fermentation process. As shown in Fig. 3, the initial VFA concentration increased with the increase in the proportion of wine vinasse. Similarly, after fermentation, the total VFA concentration showed an increasing trend with the increase in the proportion of wine vinasse.

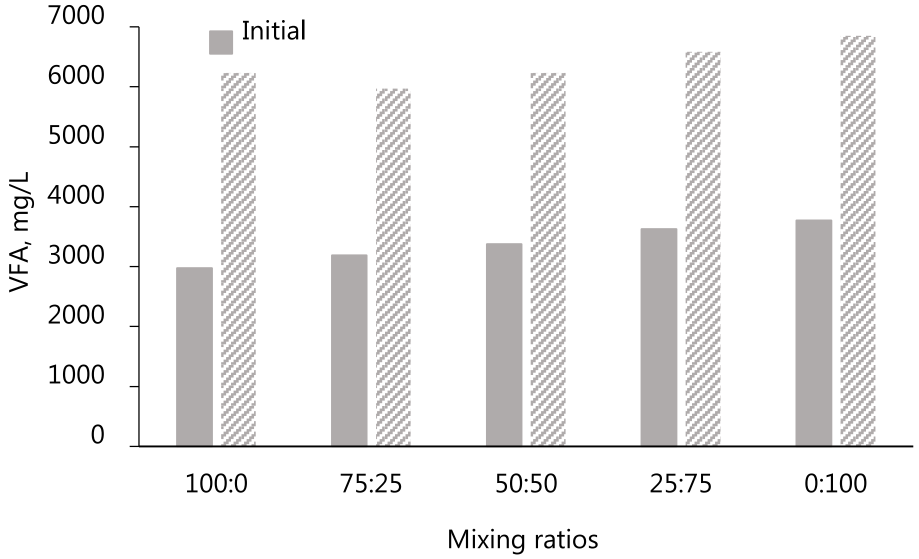

Figure 3. Change in VFA after the cofermentation of sewage sludge with wine vinasse at different mixing ratios (SS:WV).

Fig. 4 shows the composition of the main VFA obtained at the end of the test. These were acetic and butyric acids. For acetic acid, the percentages were 51.64%, 39.48%, 29.52%, 27.28% and 16.85% of total VFA at sludge:vinasse ratios of 100:0, 75:25, 50:50, 25:75 and 0:100, respectively. The other major VFA component, butyric acid, accounted for 31.95%, 47.53%, 61.98%, 64.93% and 75.61% of total VFA at sludge:vinasse ratios of 100:0, 75:25, 50:50, 25:75 and 0:100, respectively. According to the results, acetate-type fermentation was dominant in the monofermentation of sludge. Yang et al., [35] also found acetic acid to be the predominant product of sewage sludge monofermentation. However, for the tests containing wine vinasse, the results indicate that hydrogen production belonged to butyric-type fermentation. Butyric-type fermentation is considered to be one of the most effective production routes for hydrogen [16]. In fact, butyric acid was the major VFA in the fermentation of several types of substrates, such as the organic fraction of municipal solid waste [1], food waste and crude glycerol [23] and the organic fraction of municipal solid waste and sewage sludge [16].

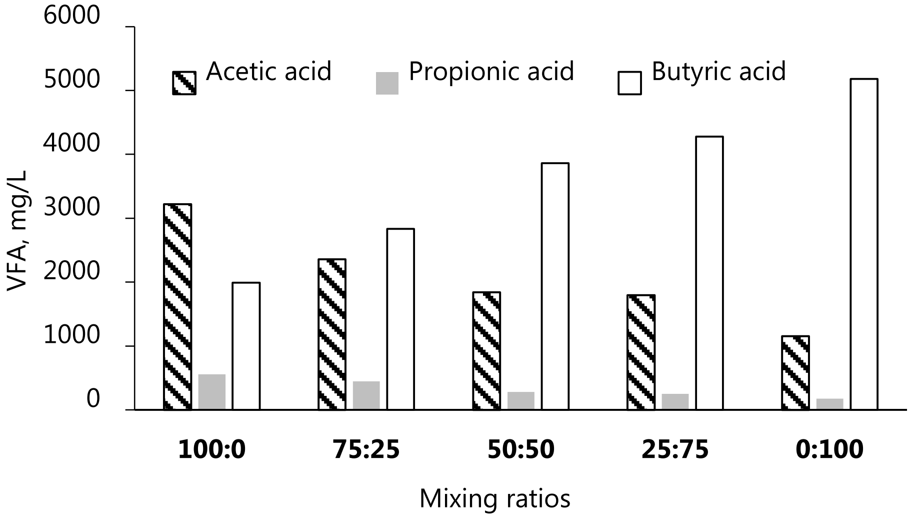

Figure 4. VFA composition after cofermentation of sewage sludge with wine vinasse at different mixing ratios (SS:WV).

An indicator normally used to evaluate the efficiency of hydrogen production is the butyrate-to-acetate concentration ratio (B:A ratio). The ratio of butyric acid to acetic acid was higher than 1 (1.2-4.5) in all tests, except for sewage sludge monofermentation (0.6). Kim et al. [36] state that higher B:A ratios and lower concentrations of propionic acid reflect higher hydrogen production efficiency. This statement is in line with our results, in which we find that the highest production of hydrogen corresponds to the highest B:A ratio.

3.3. Hydrogen production

Fig. 5 shows the cumulative hydrogen production in the cofermentation of sewage sludge with wine vinasse at different mixing ratios. The hydrogen production pattern was similar for all the tested mixtures.

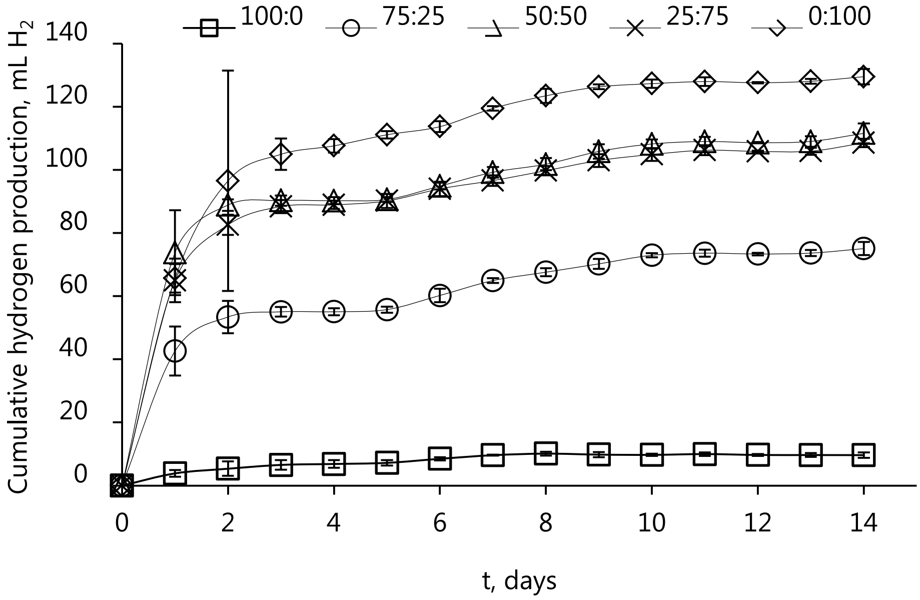

Figure 5. Cumulative hydrogen production from cofermentation of sewage sludge with wine vinasse at different mixing ratios (SS:WV).

As can be seen in Fig. 5, cofermentation of SS and WV at any mixing ratio resulted in higher hydrogen production than sludge monofermentation. Hydrogen production increased from 9.65±0.82 to 129.53±2.40 mL with the increasing proportion of WV, indicating that the large amount of organic matter in wine vinasse promoted the increase in hydrogen production. There is no significant difference ($p > 0.05$) between the hydrogen production obtained at the mixing ratios of 50:50 and 25:75. The results obtained in this study are in line with those reported by other authors for cofermentation of sludge with several substrates, such as food waste [4], fallen leaves [11] and ryegrass

[2]. In all cases, sludge cofermentation enhanced hydrogen production with respect to monofermentation.

3.4. Kinetic analysis

The modified Gompertz model is frequently used to analyse the kinetics of hydrogen production [8,21,37,38]. In order to better assess the efficiency of hydrogen evolution, two other kinetic models (the Cone and first-order kinetic models) were also applied to characterize hydrogen production in this study. Table 3 shows the results of the relevant kinetic parameters obtained for the three models. It can be seen that all three models provide a good fit to cumulative hydrogen production, with an R^2 of 0.8590-0.9521 for the modified Gompertz model, an R^2 of 0.9712-0.9947 for the Cone model, and an R^2 of 0.9040-0.9778 for the first-order kinetic model. According to the results from all three models, all the cofermentation systems gave rise to higher cumulative hydrogen production potential (P) than monofermentation of sewage sludge, the highest P in the cofermentation test being obtained at a 50:50 mixing ratio. The Gompertz and first-order kinetic models showed the highest P in the monofermentation of wine vinasse. This the biodegradability of wine vinasse and justified its use as a co-substrate in hydrogen production.

As for the maximum hydrogen production rate, this was only 0.0920mL/h for sludge monofermentation and increased for cofermentation with wine vinasse. The highest R_m value, 3.0984mL/h, was obtained at a mixing ratio of 50:50.

Model	Parameters	Mixing ratio (SS:WV)				
		100:0	75:25	50:50	25:75	0:100
Modified Gompertz	P (mL)	9.6802	66.7630	101.2758	99.3351	121.7295
	Difference (%)	0.33	11.06	9.23	8.49	6.02
	R_m (mL/h)	0.0920	1.4793	3.0984	2.4902	2.3569
	λ(h)	0	0	0	0	0
	R^2	0.9359	0.8590	0.9206	0.9333	0.9521
	Adj R^2	0.9252	0.8355	0.9073	0.9222	0.9441
	SEE (Standar Error of Estimate)	0.7921	7.7726	8.4010	7.6148	8.0792
	RMSE	0.7085	6.9520	7.5140	6.8109	7.2263
Cone	P (mL)	12.1186	157.1581	212.6054	147.4788	139.7993
	Difference (%)	20.39	52.24	47.52	26.40	7.35
	k_{hyd} (1/h)	0.0167	0.0021	0.0031	0.0282	0.0398
	n	0.9677	0.3479	0.2576	0.4455	0.9657
	R^2	0.9712	0.9850	0.9909	0.9947	0.9947
	Adj R^2	0.9663	0.9825	0.9894	0.9938	0.9938
	SEE	0.5315	2.5332	2.8442	2.1460	2.6964
	RMSE	0.4754	2.2658	2.5439	1.9194	2.4117
First-order kinetic	P (mL)	9.9671	69.1164	102.4437	100.9485	124.1789
	Difference (%)	3.20	7.92	8.19	7.00	4.13
	k_{hyd} (1/h)	0.0146	0.0044	0.0444	0.0353	0.0276
	R^2	0.9692	0.9040	0.9371	0.9571	0.9778
	Adj R^2	0.9668	0.8966	0.9323	0.9538	0.9761
	SEE	0.5275	6.1628	7.1829	5.8701	5.2903
	RMSE	0.4911	5.7372	6.6869	5.4647	4.9250

Table 3. Kinetic analysis for cofermentation of sewage sludge with wine vinasse at different mixing ratios.

Regarding lag time, the value of 0h was obtained in all tests, meaning that hydrogen was produced before one day of incubation. This rapid onset of fermentation might be due to using an inoculum that was from a termophilic active acidogenic reactor.

The first-order kinetic model showed smaller differences between predicted hydrogen production and measured hydrogen production than the other two models at all SS:WV mixing ratios and when only fermenting wine vinasse. The greatest differences between predicted hydrogen production and measured hydrogen production were obtained using the Cone model. The low error values (less than 12%) obtained for first-order kinetic and modified Gompertz models suggests the applicability of both models to predict the hydrogen yield. In contrast, the RMSE (Root Mean Square Error) values calculated for the Cone model were the lowest (0.4754-2.5439) followed by the first-order kinetic model (0.4911-6.6869) and the modified Gompertz model (0.7085-7.5140), indicating the applicability of Cone model for predict the hydrogen production. Based on these results and the values of R^2, where the best adjustments are obtained for the Cone model followed by the first-order kinectic model, can be affirmed that the modified Gompertz model was not always more precise when assessing hydrogen evolution. Yang and Wang [2,24] also reported that other models (Cone or first-order kinetic models) gave a better fit to hydrogen production than the modified Gompertz model in their studies on the fermentation of raw grass applying different pretreatment methods and in the cofermentation of sewage sludge with ryegrass, respectively.

3.5. Hydrogen yield

Hydrogen yield, defined in this study as the hydrogen production volume per gram of VS added, is a good index for evaluating the efficiency of hydrogen fermentation. Fig. 6 shows the hydrogen yields obtained for the different sludge-to-vinasse mixtures. The hydrogen yield from sludge monofermentation was only 3.17±1.28 mL H_2/g VS_{added}. As can be seen in Fig. 6, cofermentation of sewage sludge with wine vinasse enhanced the hydrogen yield, which was 26.29±2.13, 41.16±3.57 and 43.25±1.52mL H_2/g VS_{added} at mixing ratios of 75:25, 50:50 and 25:75, respectively. Furthermore, the hydrogen yield was 55.43±2.86 mL H_2/g VS_{added} for wine vinasse monofermentation. A significant increase in hydrogen yield was observed when the proportion of wine vinasse increased from 25 to 50%, although it did not improve when increasing the proportion of wine vinasse to 75%. The hydrogen yields at sewage sludge:vinasse ratios of 50:50 and 25:75 were 13 and 14 times higher than that obtained in sludge monofermentation. Yang et al., [11] achieves a similar maximum yield (37.8mL H_2/g VS_{added}) in the cofermentation of SS with fallen leaves (20:80). However, higher results are reported in the cofermentation of sludge and food waste (25:75) [39] or ryegrass (30:70) [2] with a hydrogen yield of 174.6mL H_2/g VS and 60mL H_2/g VS, respectively. Although the results are different, depending on the residue used as a cosubstrate in the fermentation of sludge, the hydrogen yield obtained through cofermentation is improved in all studies.

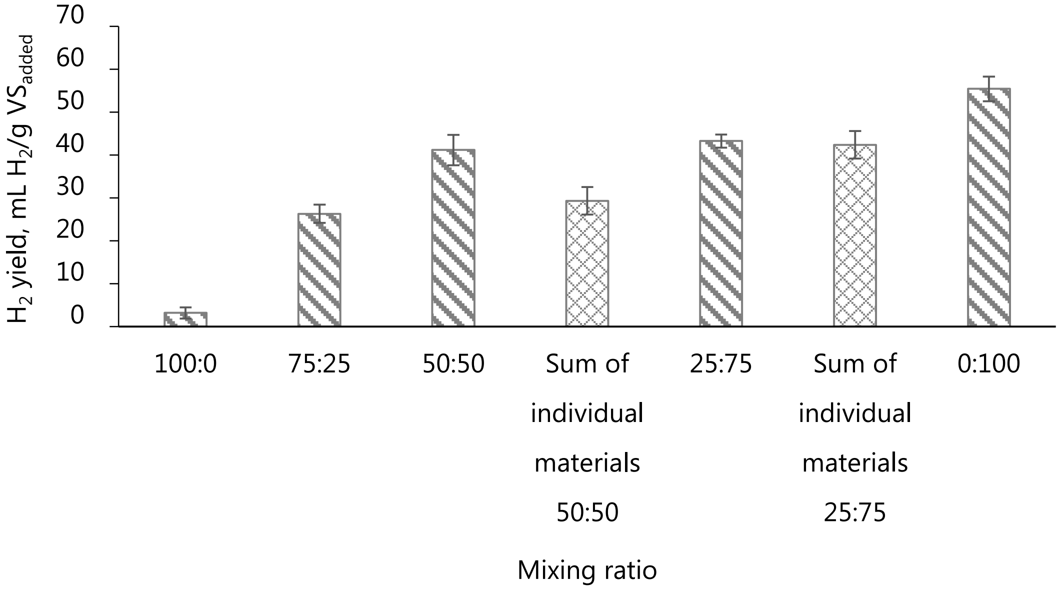

Figure 6. Hydrogen yield from cofermentation of sewage sludge with wine vinasse at different mixing ratios (SS:WV).

On the other hand, hydrogen yield obtained at sludge-vinasse ratio of 50:50 (41.16±3.57mL H_2/g VS_{added}) was 40% higher than the sum of hydrogen yield from sewage sludge and wine vinasse monofermentations multiplying by 0.5 (29.30±3.22mL H_2/g VS_{added}). Likewise, it was calculated for the sludge-vinasse ratio of 25:75, obtaining that the hydrogen yield at sludge-vinasse ratio of 25:75 (43.25±1.52mL H_2/g VS_{added}) was 2% higher than the sum of hydrogen yield of both monofermentation (42.36±3.21mL H_2/g VS_{added}) (i.e. the sum of hydrogen yield of the sewage sludge multiplying by 0.25 and hydrogen yield of the wine vinasse multiplying by 0.75). These results proved the synergistic effect of these two substrates. Other authors [2,11] also found a similar synergistic effect during hydrogen fermentation in the cofermentation of sewage sludge and fallen leaves and sewage sludge and ryegrass. The possible reason may be that the addition of wine vinasse to sewage sludge provided more suitable C/N ratio for fermentative bacteria. C/N ratio required for hydrogen fermentation was suggested to be 20-30 [11,39] and C/N ratio was only 5.10 for the sewage sludge. Thus, mixing the sewage sludge with wine vinasse (C/N ratio 25.33) increases the C/N ratio to improve hydrogen production. Aditionally, other reason could be that the used sewage sludge could contain many types of toxic compounds [40] and the addition of wine vinasse diluted these toxic that could inhibit the activity of fermentative bacteria.

4. Conclusion

This study has demonstrated that the addition of wine vinasse could significantly enhance sewage sludge hydrogen fermentation. The hydrogen yield was 41.16±3.57 and 43.25±1.52mLH_2/gVS_{added} at mixing ratios of 50:50 and 25:75, respectively, there being no significant difference between these yields. The highest VS removal was 37% when the mixing ratio was 25:75. Furthermore, the synergistic effect of cofermentation on hydrogen production was demonstrated, obtaining a 40% increase in hydrogen yield at a mixing ratio of 50:50 compared to the sum of the hydrogen production of the monofermentations of sewage sludge and wine vinasse. The Cone and first-order kinetic models provided a better fit to hydrogen production than the modified Gompertz model.

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References

[1] Zahedi S, Sales D, Romero LI, Solera R. Hydrogen production from the organic fraction of municipal solid waste in anaerobic thermophilic acidogenesis: Influence of organic loading rate and microbial content of the solid waste. Bioresour Technol 2013;129:85–91. https://doi.org/10.1016/j.biortech.2012.11.003.

[2] Yang G, Wang J. Co-fermentation of sewage sludge with ryegrass for enhancing hydrogen production: Performance evaluation and kinetic analysis. Bioresour Technol 2017;243:1027–36. https://doi.org/10.1016/j.biortech.2017.07.087.

[3] Angeriz-Campoy R, Fdez-Güelfo LA, Tyagi VK, Álvarez-Gallego CJ, Romero-García LI. New criteria to determine the destabilization of the acidogenic anaerobic co-digestion of organic fraction of municipal solid waste (OFMSW) with mixed sludge (MS). Bioresour Technol 2018;248:174–9. https://doi.org/10.1016/j.biortech.2017.05.195.

[4] Li Z, Chen Z, Ye H, Wang Y, Luo W, Chang JS, et al. Anaerobic co-digestion of sewage sludge and food waste for hydrogen and VFA production with microbial community analysis. Waste Manag 2018;78:789–99. https://doi.org/10.1016/j.wasman.2018.06.046.

[5] Yin Y, Wang J. Biohydrogen production using waste activated sludge disintegrated by gamma irradiation. Appl Energy 2015;155:434–9. https://doi.org/10.1016/j.apenergy.2015.05.105.

[6] Han SK, Shin HS. Biohydrogen production by anaerobic fermentation of food waste. Int J Hydrogen Energy 2004;29:569–77. https://doi.org/10.1016/j.ijhydene.2003.09.001.

[7] Luo G, Xie L, Zou Z, Wang W, Zhou Q. Evaluation of pretreatment methods on mixed inoculum for both batch and continuous thermophilic biohydrogen production from cassava stillage. Bioresour Technol 2010;101:959–64. https://doi.org/10.1016/j.biortech.2009.08.090.

[8] Sivagurunathan P, Kumar G, Kobayashi T, Xu K, Kim SH. Effects of various dilute acid pretreatments on the biochemical hydrogen production potential of marine macroalgal biomass. Int J Hydrogen Energy 2017;42:27600–6. https://doi.org/10.1016/j.ijhydene.2017.05.106.

[9] Rivero M, Solera R, Perez M. Anaerobic mesophilic co-digestion of sewage sludge with glycerol: Enhanced biohydrogen production. Int J Hydrogen Energy 2014;39:2481–8. https://doi.org/10.1016/j.ijhydene.2013.12.006.

[10] Kim SH, Han SK, Shin HS. Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge. Int J Hydrogen Energy 2004;29:1607–16. https://doi.org/10.1016/j.ijhydene.2004.02.018.

[11] Yang G, Hu Y, Wang J. Biohydrogen production from co-fermentation of fallen leaves and sewage sludge. Bioresour Technol 2019;285:121342. https://doi.org/10.1016/j.biortech.2019.121342.

[12] Carrilho ENVM, Labuto G, Kamogawa MY. Destination of Vinasse, a Residue From Alcohol Industry: Resource Recovery and Prevention of Pollution. Elsevier Inc.; 2016. https://doi.org/10.1016/B978-0-12-803837-6.00002-0.

[13] Kharayat Y. Distillery wastewater: bioremediation approaches. J Integr Environ Sci 2012;9:69–91. https://doi.org/10.1080/1943815X.2012.688056.

[14] Chowdhary P, Khan N, Bharagava RN. Distillery Wastewater: it's Impact on Environment and Remedies. Environ Anal Ecol Stud 2018;1:14–7. https://doi.org/10.31031/EAES.2018.01.000507.

[15] Montañés R, Pérez M, Solera R. Anaerobic mesophilic co-digestion of sewage sludge and sugar beet pulp lixiviation in batch reactors: Effect of pH control. Chem Eng J 2014;255:492–9. https://doi.org/10.1016/j.cej.2014.06.074.

[16] Tyagi VK, Angériz Campoy R, Álvarez-Gallego CJ, Romero García LI. Enhancement in hydrogen production by thermophilic anaerobic co-digestion of organic fraction of municipal solid waste and sewage sludge - Optimization of treatment conditions. Bioresour Technol 2014;164:408–15. https://doi.org/10.1016/j.biortech.2014.05.013.

[17] Pecorini I, Baldi F, Iannelli R. Biochemical hydrogen potential tests using different inocula. Sustain 2019;11:1–17. https://doi.org/10.3390/su11030622.

[18] Jia X, Wang Y, Ren L, Li M, Tang R. ScienceDirect Early warning indicators and microbial community dynamics during unstable stages of continuous hydrogen production from food wastes by thermophilic dark fermentation. Int J Hydrogen Energy 2019. https://doi.org/10.1016/j.ijhydene.2019.08.082.

[19] Dessì P, Lakaniemi AM, Lens PNL. Biohydrogen production from xylose by

fresh and digested activated sludge at 37, 55 and 70 °C. Water Res 2017;115:120–9. https://doi.org/10.1016/j.watres.2017.02.063.

[20] Balachandar G, Varanasi JL, Singh V, Singh H, Das D. Biological hydrogen production via dark fermentation: A holistic approach from lab-scale to pilot-scale. Int J Hydrogen Energy 2020;45:5202–15. https://doi.org/10.1016/j.ijhydene.2019.09.006.

[21] Van Ginkel SW, Oh SE, Logan BE. Biohydrogen gas production from food processing and domestic wastewaters. Int J Hydrogen Energy 2005;30:1535–42. https://doi.org/10.1016/j.ijhydene.2004.09.017.

[22] Silva FMS, Mahler CF, Oliveira LB, Bassin JP. Hydrogen and methane production in a two-stage anaerobic digestion system by co-digestion of food waste, sewage sludge and glycerol. Waste Manag 2018;76:339–49. https://doi.org/10.1016/j.wasman.2018.02.039.

[23] Silva FMS, Oliveira LB, Mahler CF, Bassin JP. Hydrogen production through anaerobic co-digestion of food waste and crude glycerol at mesophilic conditions. Int J Hydrogen Energy 2017;42:22720–9. https://doi.org/10.1016/j.ijhydene.2017.07.159.

[24] Yang G, Wang J. Kinetics and microbial community analysis for hydrogen production using raw grass inoculated with different pretreated mixed culture. Bioresour Technol 2018;247:954–62. https://doi.org/10.1016/j.biortech.2017.09.041.

[25] Yang G, Wang J. Enhancement of biohydrogen production from grass by ferrous ion and variation of microbial community. Fuel 2018;233:404–11. https://doi.org/10.1016/j.fuel.2018.06.067.

[26] Standard methods for the examination of water and wastewater. In: APHA, AWWA, WPCF. 22th Ed Washingt Am Public Heal Assoc 2012.

[27] Zahedi S, Sales D, García-Morales JL, Solera R. Obtaining green energy from dry-thermophilic anaerobic co-digestion of municipal solid waste and biodiesel waste. Biosyst Eng 2018;170:108–16. https://doi.org/10.1016/j.biosystemseng.2018.04.005.

[28] Montañés R, Solera R, Pérez M. Anaerobic co-digestion of sewage sludge and sugar beet pulp lixiviation in batch reactors: Effect of temperature. Bioresour Technol 2015;180:177–84. https://doi.org/10.1016/j.biortech.2014.12.056.

[29] Tena M, Perez M, Solera R. Effects of several inocula on the biochemical hydrogen potential of sludge-vinasse co-digestion. Fuel 2019;258. https://doi.org/10.1016/j.fuel.2019.116180.

[30] Zahedi S, Solera R, García-Morales JL, Ennouri H, Sales D. Evaluation of the effect of glycerol supplementation on the anaerobic digestion of real municipal solid waste in batch mode. Fuel 2017;193:15–21. https://doi.org/10.1016/j.fuel.2016.12.024.

[31] Zahedi S, Solera R, García-Morales JL, Sales D. Effect of the addition of glycerol on hydrogen production from industrial municipal solid waste. Fuel 2016;180:343–7. https://doi.org/10.1016/j.fuel.2016.04.063.

[32] Kim M, Liu C, Noh JW, Yang Y, Oh S, Shimizu K, et al. Hydrogen and methane production from untreated rice straw and raw sewage sludge under thermophilic anaerobic conditions. Int J Hydrogen Energy 2013;38:8648–56. https://doi.org/10.1016/j.ijhydene.2013.04.079.

[33] Lin Y, Wu S, Wang D. Hydrogen-methane production from pulp & paper sludge and food waste by mesophilic-thermophilic anaerobic co-digestion. Int J Hydrogen Energy 2013;38:15055–62. https://doi.org/10.1016/j.ijhydene.2012.01.051.

[34] Zhou P, Elbeshbishy E, Nakhla G. Optimization of biological hydrogen production for anaerobic co-digestion of food waste and wastewater biosolids. Bioresour Technol 2013;130:710–8. https://doi.org/10.1016/j.biortech.2012.12.069.

[35] Pan Y, Zhi Z, Zhen G, Lu X, Bakonyi P, Li YY, et al. Synergistic effect and biodegradation kinetics of sewage sludge and food waste mesophilic anaerobic co-digestion and the underlying stimulation mechanisms. Fuel 2019;253:40–9. https://doi.org/10.1016/j.fuel.2019.04.084.

[36] Kim S, Choi K, Kim JO, Chung J. Biological hydrogen production by anaerobic digestion of food waste and sewage sludge treated using various pretreatment technologies. Biodegradation 2013;24:753–64. https://doi.org/10.1007/s10532-013-9623-8.

[37] Assawamongkholsiri T, Reungsang A, Pattra S. Effect of acid, heat and combined acid-heat pretreatments of anaerobic sludge on hydrogen production by anaerobic mixed cultures. Int J Hydrogen Energy 2013;38:6146–53. https://doi.org/10.1016/j.ijhydene.2012.12.138.

[38] Rafieenia R, Pivato A, Lavagnolo MC. Effect of inoculum pre-treatment on mesophilic hydrogen and methane production from food waste using two-stage anaerobic digestion. Int J Hydrogen Energy 2018;43:12013–22. https://doi.org/10.1016/j.ijhydene.2018.04.170.

[39] Cheng J, Ding L, Lin R, Yue L, Liu J, Zhou J, et al. Fermentative biohydrogen and biomethane co-production from mixture of food waste and sewage sludge: Effects of physiochemical properties and mix ratios on fermentation performance. Appl Energy 2016;184:1–8. https://doi.org/10.1016/j.apenergy.2016.10.003.

[40] Yang G, Wang J. Biohydrogen production by co-fermentation of sewage sludge and grass residue: Effect of various substrate concentrations. Fuel 2019;237:1203–8. https://doi.org/10.1016/j.fuel.2018.10.026.