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Enhancing Hydrogen Production by Zeolite Addition in the Dark Fermentation Process of Urban Organic Waste

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One of the objectives of the European Union (EU) 2030 strategy is the sustainable growth through a series of measures focused on the application of the “Circular Economy” (ec.europa.eu, 2020) with many benefits also for climate, environment, and society. Food waste and sewage sludge management is part of this actions plan. Both streams are ideal source for microbial valorization processes to produce biofuels in a sustainable way.

Anaerobic digestion (AD) is the benchmark robust technology for biogas production; however, the dark fermentation (DF) is a higher-rate process which offers the possibility to accumulate important building blocks (volatile fatty acids; VFA) and hydrogen from renewable resources, at reduced volumetric impact compared to AD. Focusing on this goal, the effectiveness of the hydrogenase enzymes needs to be favoured; some strategies have been already adopted: maintaining pH between 5.0-6.0; limiting the activities of methanogenic bacteria, not responsible for VFA and H_2 accumulation, by decreasing the hydraulic retention time (HRT).

Mesophilic and thermophilic bio-H_2 and VFA production by DF process from food waste (alone or mixed with sewage sludge) has been studied in this work through batch assays. Pursuing the interest of studying interactions between adsorbents materials and bacteria, some trials were amended with zeolite (Z; chabazite type) at zeolite/inoculum ratio (Z/I) of 0.20 g Z/g $VS_{inoculum}$. The zeolite's presence brought the H_2 yield up to 0.04 m^3 H_2/kg VS in thermophilic trials, in parallel to the increased acetic acid production (close to 50% of the organic acids, including lactic and VFA). The rate of H_2 production also increased with Z addition, up to 27.3 mmol/(L d), 23% higher than control without Z. The positive effect of zeolite could be related to its associated surface area (available for bacterial adhesion), or ability to adsorb/exchange protons. These results open novel perspectives not only in bio-H_2 production but also in the study of interactions between bacteria and inorganic-porous materials.

1. Introduction

The European Union (EU) is setting its priorities addressed to the transition towards a circular economy by adopting the so called “closing the loop” approach within the industrial production systems. One of the objectives of this approach is to maintain the value of resources, materials and products for the longest time possible and, at the same time, minimizing the production of waste to be disposed of (European Commission 2015). Starting from waste, their conversion into added-value products can be realized in a sustainable manner with biological processes, according to the organic content (volatile solids, VS) and to specific production process in which waste has its origin. The use of renewable resources such as biomasses or even organic waste as source for a new generation of added-value products is a challenge since the competition with fossil fuel resources in the traditional production process in the is still high. End-product identification and cost-effecting schemes are key

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elements in the design of biological processes and organic waste valorization; these factors can face effectively the competition (Zabaniotou et al. 2019).

High-rate dark fermentation (DF) process includes only the first metabolic pathways of the anaerobic digestion (AD); here, the organics' degradation occurs along with the production of interesting intermediate metabolites, among others volatile fatty acids (VFA) and hydrogen (Gottardo et al., 2023). Compared to the traditional fossil-fuel based technologies, the interest in the hydrogen production is mainly driven by its high energy yield (142.35 kJ/g) and the zero carbon emissions. Despite of these advantages, its production from waste is not yet established as industrial scale technology due to the low rate of hydrogen production, which in turn depends on the fast hydrogen consumption by some CO_2-reducing methanogens (if high HRT is adopted) and to the low degradation rate of some waste feedstock (especially those ones with low carbohydrate content). To better address such issues, the operating conditions of the DF processes need to be adapted to each specific feedstock, especially when subjected to a certain variability over the year (food waste as an example). Both food waste and sewage sludge are excellent raw material for hydrogen recovery since they are characterized by a high organic matter concentration and a continuous production in municipalities. Apart from hydrogen, the produced VFA within the DF process are valuable building blocks for the chemical industry or biopolymer synthesis (Villano et al., 2010; Valentino et al., 2013). To sustain the fermentability of sewage sludge, generally richer in proteins and, in turn, slower biodegradable than food waste, some researchers recommend mixing sludge with other co-substrates for an improved nutritional balance (C/N ratio) and putrescence features (Vidal-Antich et al., 2021).

This study has been developed to assess the effect of the feedstock composition and temperature on the main mechanisms responsible for the hydrogen accumulation, with a particular insight on the VFA spectrum change during the performed batch tests. Ethanol, lactic acid and VFA have been monitored in the liquid phase whereas hydrogen has been quantified in the gas phase to evaluate its production rate and yield. Parallel batch tests have been conducted with the addition of zeolite (Z, chabazite type), at a content of 0.20 g Z/g $VS_{inoculum}$ (Silva et al., 2021), to investigate its effect on the hydrogen production rate and yield as well as on VFA dynamics.

2. Materials and Methods

The sewage sludge (SS) was taken from the static thickener of the municipal WWTP of Treviso (northeast Italy). Food waste (FW) was recovered from the separate collection of more than 50 districts in Treviso province, subjected to a mechanical trituration, inert removal, squeezing and homogenization in an external plant. Both streams have been utilized separately and mixed in a series of batch DF tests. Sludge and food waste have been characterized for total and volatile solids (TS and VS), soluble chemical oxygen demand (COD_{SOL}), VFA, ammonia, phosphate, Total Kjeldahl Nitrogen (TKN) and organic phosphorus (P) (Table 1). The zeolite was made of 64% w/w chabazite, with a water retention of 37.8%; the 98% of the mineral material had less than 0.15 mm. For metal analysis, the samples were initially disintegrated through acid digestion and the digested samples were analysed using an ICP-MS NexION 350X (PerkinElmer) coupled with the seaFAST autosampler (ESI). Data are available in Tuci et al., 2024.

Table 1: Main Parameters of sewage sludge and food waste streams

Parameters	Unit	Sewage Sludge (SS)	Food Waste (FW)
TS	g/L	28.0 ± 0.5	49 ± 3
VS	g/L	22.6 ± 0.3	42 ± 2
COD_{SOL}	g/L	0.28 ± 0.02	16 ± 2
COD_{VFA}	g/L	-	4.1 ± 0.2
pH	-	7.1 ± 0.3	5.3 ± 0.6
Ammonia	g $N\text{-}NH_4^+$/L	0.45 ± 0.06	0.12 ± 0.02
Phosphate	g $P\text{-}PO_4^{3-}$/L	0.09 ± 0.02	0.02 ± 0.01
TKN	g N/kg TS	37 ± 2	16 ± 2
P	g P/kg TS	4 ± 1	0.6 ± 0.1

2.1 DF batch tests

Each DF tests were conducted in duplicate in the Nautilus BMP system designed by Anaero Technology. Each bottle (0.8 L working volume) was stirred by a central mechanical stirrer and placed in a water bath, which was maintained at 37°C and 55°C for mesophilic and thermophilic conditions respectively. Both SS and FW were concentrated by centrifugation (4.500 rpm for 5 min) and then diluted with tap water to reach the VS content of 80 g/L. This step was performed to have the same initial VS level in all the DF tests, independently from the adopted temperature and/or the feedstock composition. No external inoculum was added; the tests were performed by using the fermentation capacity of the mixed consortia already present in both streams. Liquid samples were periodically taken by using an external syringe connected to a sampling port of the system; to monitor the produced gas (H_2 in particular), the gas bags were connected to the same sampling port with a three-ways valve. The following Table 2 depicts all the tests performed in the two mesophilic and thermophilic regimes; SS and FW are indicated in each test (where present).

Table 2: Summary of the operating conditions investigated in the batch tests and the assigned names.

Operating conditions	Dark Fermentation Test											
	SS^{m}	SS-FW^{m}	FW^{m}	SS(z)^{m}	SS-FW(z)^{m}	FW(z)^{m}	SS^{t}	SS-FW^{t}	FW^{t}	SS(z)^{t}	SS-FW(z)^{t}	FW(z)^{t}
Temperature (°C)	37	37	37	37	37	37	55	55	55	55	55	55
Inoculum (g VS/L)	80	80	80	80	80	80	80	80	80	80	80	80
FW content (%, v/v)	0	65	100	0	65	100	0	65	100	0	65	100
SS content (%, v/v)	100	35	0	100	35	0	100	35	0	100	35	0
Zeolite (g Z/g VS)	0	0	0	0.2	0.2	0.2	0	0	0	0.2	0.2	0.2

2.2 Analytical Methods

Except for VFA, the features depicted in Table 1 for both feedstock were quantified according to Standard Methods (APHA/AWWA/WEF, 2012). The reactors' effluent was monitored two times per week approximately for COD, pH, VFA and caproic acid, ethanol and lactic acid. The quantification of ethanol, VFA and caproic acid was conducted using AGILENT 6890N gas chromatograph equipped with a flame ionization detector (at 200°C), a fused silica capillary column, DB-WAX (15 m x 0.53 mm × 0.5 µm film thickness); hydrogen was the gas carrier. The chromatographic run was conducted by increasing the temperature from 40 to 200°C, at a rate of 10°C/min. The samples were analysed before being centrifuged and filtered (0.2 µm filter porosity), as done for lactic acid quantification, which was performed by using Megazyme kits (Megazyme, 2018). The H_2 percentage was quantified with the same GC described above, equipped with HP-PLOT MOLESIEVETM column (30 m x 0.53 m ID x 25 µm film thickness), using a thermal conductivity detector (TCD) at 250°C. The temperature of the injector was maintained at 120°C and 70 kPa; samples were taken using a gas-type syringe in 200 µL biogas amounts. The analyses were conducted at 40°C for 8 min, with Argon as gas carrier.

3. Results and discussion

3.1 Mesophilic dark fermentation of sewage sludge and food waste

The mesophilic tests highlighted the importance of the FW as co-substrate. In fact, as depicted in Figure 1A, the VFA concentration substantially increased in the mixed feedstock (FW and SS) and with FW only compared to the tests conducted with SS only. The reduced biodegradability of the sludge as well as its lower potential (compared to FW) to accumulate VFA are known factors discussed in a previous study (Moretto et al., 2019). However, the possibility to recover H_2 from the gas phase was not discussed. SS alone led to an average VFA concentration between 10-13 g COD/L, not particularly improved in the case of zeolite addition. Concerning the tests with FW only and with the FW-SS mixture, the acidification performances deeply improved despite of the same initial VS level (80 g VS/L): the final VFA concentration ranged between 20 and 25 g COD/L, with a maximum conversion yield of 0.39 g COD_{VFA}/g VS. In these tests, lactic acid was also detected, presumably derived from FW only. Considering the FW^{m} and FW(z)^{m} tests, lactic acid was detected up to 12 g COD/L; however, after an initial production, its concentration progressively decreased until the end of the tests (from 58% COD/COD of the total organic acids to less than 10% COD/COD), being replaced by acetic and butyric acid, which showed an increase from 10% to 40% COD/COD (roughly). In general, the zeolite addition sustained the acidification performances, mostly when FW is present in the feedstock, demonstrated by the higher VFA level obtained in the tests FW(z)^{m} and SS-FW(z)^{m} (25.0 and 24.2 g COD_{VFA}/L respectively) compared to FW^{m} and SS-FW^{m} (22.3 and 22.7 g COD_{VFA}/L respectively).

The increase of the VFA level, and in particular of the acetic acid in the tests where FW was present, matched with the higher H_2 production activity observed in the tests where zeolite was added. Zeolites is an aluminosilicate material which have been extensively investigated in bioprocesses (mainly anaerobic digestion) due to its ion exchange capacity, high porosity and surface area (Silva et al., 2021). In the frame of anaerobic

DF, the effect of zeolites on bio-H_2 production was certainly linked to its ion-exchanger property; in addition, zeolite can act as promoter for ammonia reduction, which is another factor that can improve the feedstock utilization by reducing its inhibitory effects along with the adsorption of other cations like Ca^{2+} and Mg^{2+} (Silva et al., 2021). The average bio-H_2 production rate was substantially increased by zeolite addition, even utilising SS only, which is a substrate with high proteins content (>40% w/w) and, in turn, less suitable for H_2 production (Johnravindar et al., 2021): 10.8 vs 19.0 mmol H_2/(L d) in the tests SS^m and $SS(z)^m$ respectively; 15.2 vs 19.6 mmol H_2/(L d) in the tests $SS\text{-}FW^m$ and $SS(z)\text{-}FW^m$ respectively; 18.5 vs 22.2 mmol H_2/(L d) in the tests FW^m and $FW(z)^m$ respectively (Figure 1B).

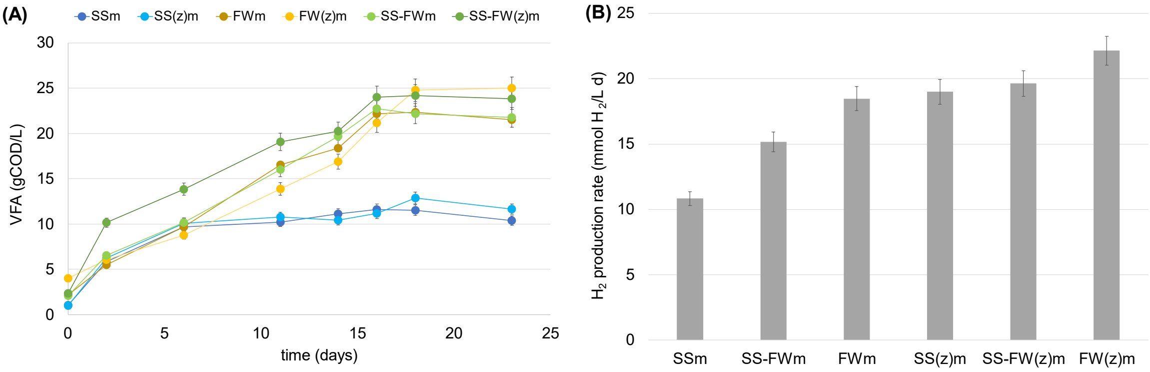

Figure 1: Trend of VFA (lactic acid not included) over time (A) and volumetric hydrogen production (B) in the mesophilic (37°C) DF test.

3.2 Thermophilic dark fermentation of sewage sludge and food waste

Thermophilic environment increases the capability of the biomass to convert the organic matter into VFA compared to mesophilic temperature. Lactic acid was also produced in the tests conducted with FW (alone or in mixture with SS) up to 15 g COD/L. However, as observed in mesophilic trials, the lactic acid concentration tended to progressively decrease over time, being replaced by acetic and butyric acid production.

Regarding the use of SS only, no lactic acid production was observed and the maximum VFA level achieved was close to 15 g COD/L (Figure 2A). Zeolite addition seemed to be not affecting the VFA production, even though a remarkable increase in the volumetric bio-H_2 production was observed (18.7 vs 24.5 mmol H_2/(L d), respectively in SS^t and $SS(z)^t$ tests).

As indicated in the previous paragraph, the FW presence is pivotal to increase the VFA concentration. Given the same initial VS level in each test, the higher VFA concentrations obtained in the thermophilic tests corresponds to higher VFA conversion yields (up to 0.48 g COD_{VFA}/g VS). It is noteworthy that zeolite addition substantially enhanced the VFA production when FW was utilized alone or in in FW-SS mixture; on the contrary, in the mesophilic trials, the effect of zeolite addition on VFA production was less remarkable. Thermophilic tests showed that VFA concentration increased up to 11% and 30% respectively in $SS\text{-}FW(z)^t$ and $FW(z)^t$, compared to the same tests conducted without zeolite addition. Having high VFA concentration in a fermented stream is a crucial factor for the valorization of a waste stream. The downstream processes to concentrate and/or separate VFA in the fermentation liquor is a factor that has to be considered to sustain VFA marketability. Adsorption techniques as well as extraction, distillation or membrane-based technologies can be less impacting from the economical point of view with highly concentrated VFA in fermentation broths (Aktij et al., 2020). In this frame, thermophilic bioprocesses enhanced the feedstock degradation and the utilization of the organic matter from fermentative consortia, producing a VFA-rich stream in the range 23-30 g COD_{VFA}/L, when FW was utilized.

Regarding the production of bio-H_2 in thermophilic trials, its volumetric rate generally increased when zeolite was added. The performance with SS was strongly improved: in the test $SS(z)^t$, the volumetric H_2 production rate (24.5 mmol H_2/(L d)) was 30% higher than value obtained in the test without zeolite addition (SS^t). The highest rate was obtained with FW only: 27.3 mmol H_2/(L d), test $FW(z)^t$. This value was 23% higher than H_2 production rate obtained in the corresponding mesophilic test ($FW(z)^m$). However, considering both series of tests (mesophilic and thermophilic), the highest increase of H_2 production rate (76%) due to the zeolite addition was obtained with SS only, under mesophilic environment.

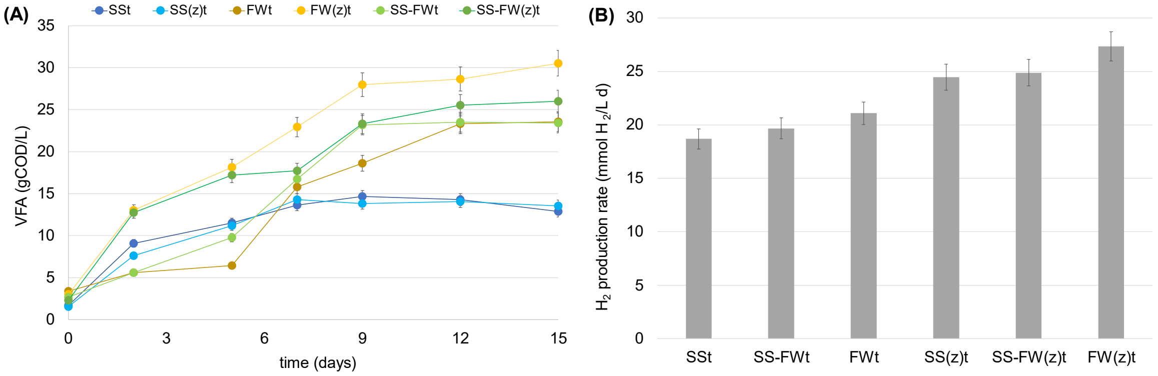

Figure 2: Trend of VFA (lactic acid not included) over time (A) and volumetric hydrogen production (B) in the thermophilic (55°C) DF test.

3.3 Preliminary considerations on hydrogen production

It is generally known that H_2 production in dark fermentation process requires low hydraulic retention time (HRT) due to the high growth rates of H_2-producer consortia (Gottardo et al., 2023). The type of feedstock also affects the H_2 production, especially when the protein/carbohydrate content is substantially different or if the substrate has been subjected to a certain pretreatment procedure. In this study, the batch tests did not allow to consider the effect of the HRT, but the feedstock composition, as well as the zeolite addition, had a certain effect on the hydrogen production. The presence of SS in the mixture certainly decreased the potentiality of hydrogen accumulation compared to what has been observed with FW only. This is a consequence of the low rate of the sludge disintegration, which in turn was due to the presence of cell's membrane and/or extracellular polymeric substances. Zeolite probably favored the feedstock disintegration with a positive impact on H_2 production, especially when SS was utilized as the only carbon source. Considering the chemical reactions associated with the acidification of the organic matter (especially sugar-rich feedstock), the production of acetic acid is stoichiometrically associated to higher H_2 accumulation, rather than butyric acid (Gottardo et al., 2023). Accordingly, at similar temperature and feedstock composition, lower butyrate/acetate ratio (COD/COD) was reflected by higher H_2 production rate (Table 3).

Table 3: Summary of the main results and performances in the batch tests.

Parameter	Dark Fermentation Test											
	SS^m	SS-FW^m	FW^m	SS(z)^m	SS-FW(z)^m	FW(z)^m	SS^t	SS-FW^t	FW^t	SS(z)^t	SS-FW(z)^t	FW(z)^t
VFA (g COD/L)	11.6	22.8	22.4	12.9	24.2	25.0	14.7	23.5	23.6	14.1	26.0	30.5
Ethanol (g COD/L)	0.53	1.09	2.81	0.23	1.98	0.77	0.55	2.10	1.91	0.36	0.88	1.66
Lactic Acid (g COD/L)	1.22	2.02	2.06	1.27	1.11	1.50	0.81	2.12	5.32	0.82	1.72	4.42
Butyric/Acetic (COD/COD)	12.70	10.50	7.96	8.37	7.50	3.72	10.20	8.44	5.79	6.21	5.45	1.63
Yield (Y_{VFA}) ($gCOD_{VFA}$/gVS)	0.18	0.36	0.35	0.20	0.38	0.39	0.23	0.37	0.37	0.22	0.41	0.48
H_2 production rate (mL H_2/L d)	10.8	15.2	18.5	19.0	19.6	22.2	18.7	19.7	21.1	24.5	24.9	27.3

4. Conclusions

The addition of natural zeolite (in the form of chabazite) improved the rates of bio-H_2 production by dark fermentation of complex feedstock such as sewage sludge, food waste and a mixture of them. Considering all the conducted batch tests, the production of other fermentative end-products rather than acetic and/or butyric acid by the mixed microbial consortium led to lower H_2 production activity comparing with the tests where acetic and butyric acids were more predominant. In addition, zeolite addition had a positive impact on H_2 production under both mesophilic and thermophilic environment, and particularly with SS used as single substrate. The presence of zeolite needs to be further evaluated in continuous operation, to better understand the bacteria interaction with its porous structure, the ion-exchanger capacity in fermentation processes and possible links between the selected microbial community with fermentation products.

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