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Fish waste valorization route: production of biohydrogen and volatile fatty acids under different process conditions

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Abstract. Acidogenic fermentation with anaerobic microbial consortia is an attractive route for fish waste (FW) valorization towards high-value products, including volatile fatty acids (VFAs) and biohydrogen (H_2). Nonetheless, literature on the influence of operational parameters on VFAs and H_2 production from FW is scarce. This study aimed to explore the influence of initial pH, food-to-microorganisms' (F/M) ratio, and co-fermentation with fruits and vegetables waste (FVW) on VFAs and H_2 production from FW. The results revealed that adjusting the initial pH to 4.5, 5.0, 6.0, 6.5, and 7.0 significantly affected VFAs and H_2 yield in comparison to the condition without initial pH adjustment ($p < 0.05$). The highest H_2 yield was observed at initial pH 4.5, reaching 59.51 ± 3.02 NmL H_2/g VS while the highest VFAs production was observed at initial pH 7, reaching a concentration of 1876 mg HAc/L. Increasing the F/M ratio from 1 to 5 resulted in a 64% increase in VFAs yield at initial pH 7. Moreover, co-fermentation with 75% FVW resulted in a 68% increase in H_2 yield, while the optimum VFAs yield (758.6 mg Hac/g VS) was obtained by co-fermentation with 50% FVW. The results are useful for the emerging trash-to-cash concept.

Keywords: co-fermentation, resource recovery, circular economy, food/microorganisms' ratio, pH

1. Introduction

Fish is an essential part of human dietary intake, serving as a source of high-quality protein, essential fatty acids, vitamins, and minerals for over 1 billion people (Byrd et al., 2021). Fish leftovers and processing byproducts, including non-edible parts such as viscera, bones, and damaged whole fish, constitute up to 70% of the original fish (Maqsood et al., 2024). Due to its negative environmental impacts when not handled properly and relevance in the achievement of a circular economy, resource recovery from fish waste (FW) has gained attention in recent years. Acidogenic fermentation with anaerobic microbial consortia is an attractive route for fish waste valorization towards value-added products, including volatile fatty acids (VFAs) and biohydrogen (H_2) (Arhin et al., 2023; Zhao et al., 2021). However, literature on the influence of operational parameters on VFAs and H_2 production from FW is scarce. This study aimed to investigate the effect of pH, food-to-microorganisms' (F/M) ratio, and co-fermentation with fruits and vegetables waste (FVW) on VFAs and H_2 production from FW.

2. Materials and methods

The FW was collected from a fish market in Pozzuoli (Napoli, Italy). The FVW was prepared by simulating the typical waste produced in Mediterranean wholesale markets, characterized by 36% of fruit waste and 64% of vegetables waste (weight basis). The inoculum used was sewage sludge from the municipal wastewater treatment plant of Nola (Napoli, Italy). The sludge was acclimatized at 35 °C for 50 d and thermally pretreated at 105 °C for 4 hours to inhibit methanogens.

Three sets of batch experiments were performed using 500 mL glass bottles (Schott Duran, Germany) under mesophilic conditions (35 °C), as summarized in **Table 1**. In the first set, seven different initial pH conditions of 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and uncontrolled pH were investigated. Afterwards, the effects of F/M ratio of 1 and 5 at initial pH of 4.5 and 7.0 were examined. In the third set, the influence of co-fermentation with FVW at different proportions was also investigated. The H_2 and VFAs production were monitored daily following standard protocols as described in Arhin et al., (2023).

Table 1. Experimental setup for the batch acidogenic fermentation experiments.

Test	Substrate	Initial pH	F/M ratio (gVS/gVS)
Set 1	FW	Uncontrolled, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0	1
Set 2	FW	4.5 and 7.0	1 and 5
Set 3	FW (100%, 50%, 25%), FVW (0%, 50%, 75%)	Uncontrolled	1

3. Results and discussion

3.1 Effect of initial pH on H_2 and volatile fatty acids production from fish waste

Adjusting the initial pH to 4.5, 5.0, 6.0, 6.5 and 7.0 significantly affected H_2 yield ($p < 0.05$). Compared to the condition without pH adjustment (19.71 ± 5.41 NmL H_2/g VS), the H_2 yield at initial pH 4.5, 5.0, 6.0, 6.5, and 7.0 were 59.51 ± 3.02, 46.45 ± 16.31, 43.03 ± 5.87, 51.22 ± 11.97, 51.33 ± 4.98, and 45.88 ±7.62 NmL H_2/g VS, respectively. As depicted in **Figure 1A**, the VFAs detected were acetic acid, propionic acid, butyric acid, and isovaleric acid. The main VFA was acetic acid, followed by propionic acid. Butyric acid was not detected when the initial pH was below 6. Also, isovaleric acid was not detected at initial pH of 4.5 and 6.5. Overall, the total VFAs concentration increased with increasing pH. Specifically, the lowest total VFAs concentration of 987 mgHAc/L was observed at initial pH of 4.5 while, the highest concentration of 1876 mgHAc/L was obtained at initial pH of 7.

3.2 Effect of initial pH and F/M ratio on H_2 yield and volatile fatty acids production from fish waste

When the F/M ratio was increased from 1 to 5 gVS/gVS at the initial pH of 4.5 and 7.0, higher H_2 yields were observed at initial pH 4.5 compared to 7.0. The H_2 yields at initial pH 4.5 were 31% and 27% higher than those of initial pH 7 for F/M ratio of 1 and 5, respectively ($p < 0.05$). In contrast, at the same F/M ratio, higher VFAs concentrations were observed at initial pH 7 compared to initial pH 4.5. As shown in **Figure 1B**, initial pH 7 was characterized by higher acetic acid concentration and higher total VFAs production compared to initial pH 4.5. These findings were more evident by increasing the F/M ratio from 1 to 5 (i.e., +8.6% for F/M=1 and +63.8% for F/M).

3.3 Effect of co-digestion on H_2 yield and volatile fatty acids production

The results of the first two sets of experiments showed that FW was a low H_2-producing substrate. Therefore, the effect of adding carbohydrate-rich FVW on the acidogenic fermentation process was examined. As expected, the highest H_2 yield of 87.98 ± 10.2 NmL H_2/g VS was obtained by co-fermenting 25% of FW with 75% of FVW. Interestingly, for the soluble metabolites, co-fermenting 50% of FW and 50% of FVW promoted the total production of acids and ethanol (**Figure 1C**), with a yield of 758.6 mg Hac/g VS. These results highlight the positive effect of a well-balanced substrate on the activities of the microbiome.

4. Conclusions

This study investigated the effect of initial pH, F/M ratio, and co-fermentation on VFAs and H_2 production from FW. The initial pH significantly influenced VFAs and H_2 yield. At the same F/M ratio, a low initial pH led to higher H_2 yeild while a high intial pH promoted VFAs production. Co-fermenting FW and FVW at an equal ratio led the the highest VFAs yield while a higher FVW content promoted H_2 production. The results are useful for valuable biobased products generation from FW.

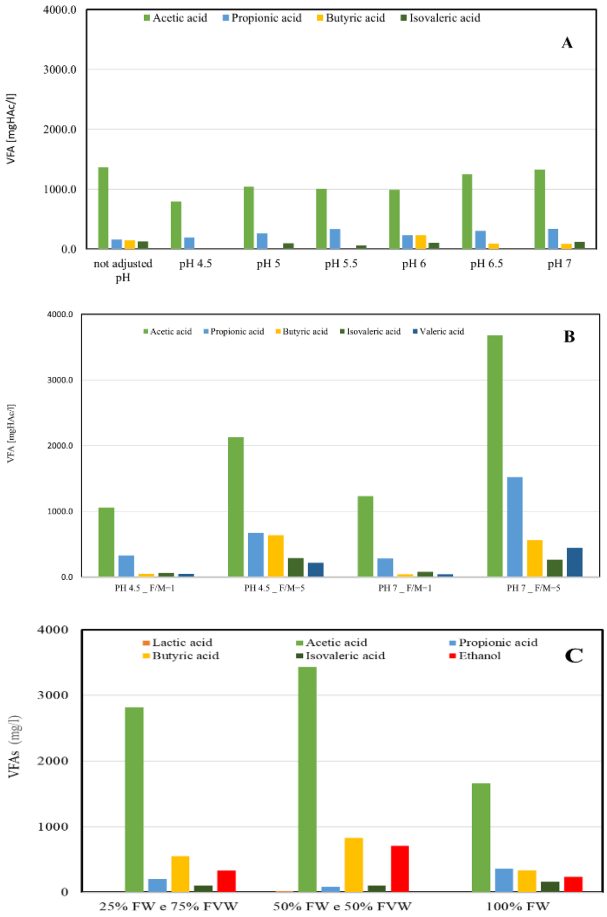

Figure 1. VFAs production from FW (A) at different initial pH levels, (B) initial pH of 4.5 and 7 at F/M ratio of 1 and 5, and (C) co-fermentation with FVW.

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