Coupling fish waste anaerobic digestion, ammonia extraction, and microbial protein production

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Abstract: This study investigates coupled fish waste anaerobic digestion (AD), electrochemical ammonia extraction, and microbial protein production using hydrogen-oxidizing bacteria (HOB). AD of fish waste after protein extraction was performed in a lab-scale continuously stirred-tank reactor (CSTR) reaching a maximum methane yield on volatile solids (VS) of 404 $mL \cdot g^{-1}$. Online electrochemical ammonium extraction achieved 14 $mg \cdot L^{-1} \cdot d^{-1}$ extraction rate. Extracted ammonium served as a nitrogen source for HOB cultures, which exhibited growth rate: 0.97 d^{-1}; cell dry weight (CDW) yield on H_2: 2.1 g g^{-1}; protein content on CDW: 28.01 g g^{-1}. Flue gas impurities (CO, NO_2, SO_2) had overall minimal effects on HOB growth and yields. Ongoing efforts focus on process optimization by modifying electrochemical cell configuration and on investigation of continuous HOB cultivation.

Keywords: anaerobic digestion; fish waste; electrochemical ammonia extraction; microbial protein; hydrogen oxidizing bacteria.

Introduction

Tackling the risks related to environmental deterioration and resources insecurity involves reshaping industrial systems by upcycling waste streams. Food and animal feed production systems should be thus urgently optimized targeting reduced emissions and improved circularity. A novel, potentially low-carbon and low-cost upcycling streamline for organics- and nitrogen-rich waste streams of fishmeal production industries consists of AD coupled with electrochemical ammonia extraction and microbial protein production by HOB. The extracted ammonia can be used as nitrogen source, while flue gases provide CO_2 for HOB growth, being renewable H_2 their energy source. Nonetheless, several challenges have to be overcome during process upscaling, which we address in this study. These include (a) assessing the stability and biomethane yield of AD with fish processing wastewater as substrate; (b) optimizing biomethane productivity and ammonia recovery; and (c) assessing the effects of flue-gas impurities (e.g., CO, NOx, SOx) on the growth rate, protein yield and quality of HOB cultures.

Material and Methods

Industrial fish waste after protein extraction (TS 8.3 ± 0.06 %; VS 7.02 ± 0.04 %; Total VFA 25 ± 1 $g \cdot L^{-1}$; Total N 14.82 ± 1.5 $g \cdot L^{-1}$) was digested at mesophilic conditions in a continuous CSTR (active volume 1.9 L). The biomethane yield and productivity, as well as the reactor performance stability, were assessed at HRT of 15 d and at organic loading rates (OLR) between 0.8 and 1.2 $g_{VS} \cdot L^{-1} \cdot d^{-1}$. During a subsequent operational stage, the digestate was continuously recirculated through the anodic chamber of an electrodialysis cell. The electrodialysis cell was designed with two separate chambers (each with a volume of 300 mL), respectively for anode and cathode, separated by a cation exchange membrane. Anode (IrO_2) and cathode

(stainless steel) had a projected area 8 cm^2. A voltage of 1.5 V was applied. Sodium carbonate (50 mM) was chosen as catholyte and 24 h was set as the catholyte retention time.

Mixed HOB cultures were cultivated in triplicate batch assays at mesophilic conditions (T= 37 °C) using standard media and the exhaust catholyte solution as nitrogen source for microbial growth. The gas composition was defined as a mixture of H_2, CO_2, O_2, N_2 (H_2/O_2 =3.3; H_2/CO_2=4.5). The total pressure of the gas phase mixture was set at 1.5 bar. Three additional sets of triplicate cultures were prepared analogously, respectively adding 0.2 bar of CO, 10 $mg \cdot L^{-1}$ NO_2, and 10 $mg \cdot L^{-1}$ SO_2.

Results and Discussion

Biogas productivity increased with increasing OLR reaching up to 700 ± 50 $mL \cdot L^{-1} \cdot d^{-1}$ (Figure 1), with biomethane yield 404±24 $mL \cdot g_{VS}^{-1}$ at OLR 1.2 $g \cdot L^{-1} \cdot d^{-1}$ and HRT 15 d, corresponding to 70 % of the yield obtained in BMP test. The ammonial nitrogen concentration stabilized on an average value of 1.8 ± 0.1 $g \cdot L^{-1}$ at OLR 1.2 $g_{VS} \cdot L^{-1} \cdot d^{-1}$ and HRT 15 d. After approximately 3 HRT both biogas productivity and biomethane productivity started declining due to VFA accumulation. Introducing electrochemical ammonium extraction with digestate recirculation in the anodic chamber (at day 110), resulted in an averaged extraction rate equivalent to 14 ± 5 $mg\text{-}N \cdot L^{-1} \cdot d^{-1}$ which led to stabilize the ammonia nitrogen concentration at 1.7 ± 0.1 $g \cdot L^{-1}$ (Figure 2). During AD-electrochemical N extraction VFA continued accumulating up to 7.1 ± 0.1 g L^{-1} while the bigas productivity declined further down to 300 ± 50 mL $L^{-1}d^{-1}$. Current work focuses on optimizing biomethane productivity, ammonia extraction rate, and VFA concentration in a three-chamber cell (anodic chamber, digestate recirculation chamber and cathodic chamber separated respectively by an anion and cation exchange membranes).

The Control HOB batch cultures fed with extracted NH_4 showed a growth rate of 0.97±0.04 d^{-1} and biomass yield 2.10±0.17 $g_{CDW} \cdot g_{H2}^{-1}$ (Table 1). Carbon monoxide at 0.2 bar initially lowered the growth rate by 20 %, though over three weeks the culture adapted. On the other hand, NO_2 and SO_2 had no significant effects, though in absence of appropriate buffering medium the presence of NO_2 and SO_2 represent an additional acidifying factor which can potentially lead to pH-related inhibitions. The obtained protein content and quality for Control, NO_2, and SO_2 exposed cultures was similar to soybean meal, while CO-exposed cultures yielded significantly lower protein content (Table 2 and Figure 2).

Conclusions

Overall, this study provided a first assessment of the performance of integrated fish waste AD with online nitrogen extraction. Ongoing work is focusing on the optimization of biogas and ammonia productivity by varying cell voltage and configuration. Furthermore, the effects of NO_2, and SO_2 on HOB growth rate and yields and protein quality were found to be not significant. CO impacted mainly the protein yield. Ongoing work is also focusing on optimizing microbial protein productivity in a continuous flat-sheet membrane reactor.

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Figures and Tables

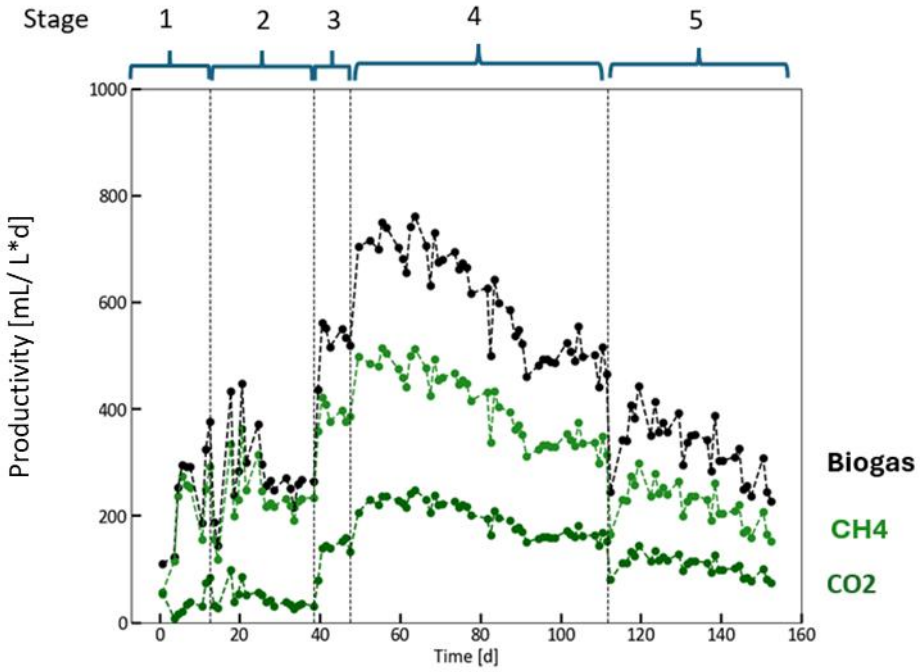

Figure 1 Specific biogas, CH_4, and CO_2 productivities over different operation stages (During Stage 1 to 4 the OLR was incremental, namely 0.4, 0.6, 0.9, 1.2 $g_{VS} \cdot L^{-1} \cdot d^{-1}$; during Stage 5 OLR was 1.2 $g_{VS} \cdot L^{-1} \cdot d^{-1}$ and online electrochemical NH4-N extraction was performed at 1.5 V).

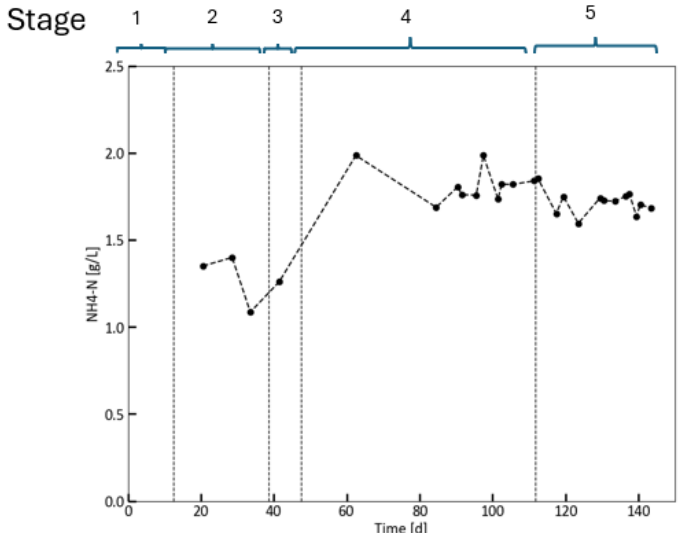

Figure 2 Ammonial nitrogen concentration over different operation stages (During Stage 1 to 4 the OLR was incremental, namely 0.4, 0.6, 0.9, 1.2 $g_{VS} \cdot L^{-1} \cdot d^{-1}$; during Stage 5 OLR was 1.2 $g_{VS} \cdot L^{-1} \cdot d^{-1}$ and online electrochemical NH4-N extraction was performed at 1.5 V).

Table 1 Growth rate, H_2 uptake rate and CDW yield on H_2 of HOB batch cultures on the third cultivation week

Condition	Growth rate (d^{-1})	H_2 uptake rate (mg H_2 d^{-1})	Y_{CDW/H_2} (g CDW g H_2^{-1})
Control	0.97 ± 0.04	3.96 ± 0.28	2.10 ± 0.17
CO	1.54 ± 0.13	5.31 ± 0.85	2.18 ± 0.11
Nitrite	0.96 ± 0.10	5.75 ± 0.31	1.72 ± 0.16
Sulfite	0.81 ± 0.06	7.35 ± 0.71	2.34 ± 0.13

Table 2 Protein content of CDW of HOB batch cultures after the third cultivation week

Batch	Total protein (% CDW)
Soybean meal	34.33 ± 1.02
Menhaden fish meal	47.86 ± 1.04
Control	28.07 ± 1.25
CO	14.46 ± 1.56
Nitrite	29.84 ± 5.60
Sulfite	30.81 ± 1.20

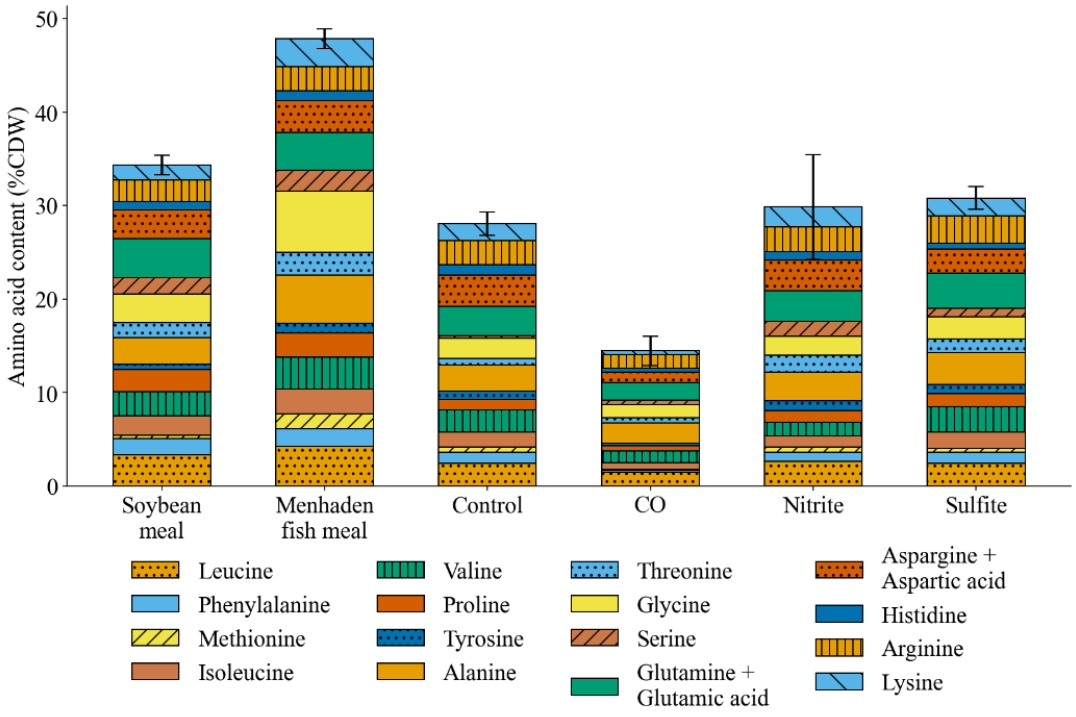

Figure 3 Amino acid profile of protein content (HOB batch cultures after the third cultivation week).