Food waste served three ways: butyric, lactic or caproic acid

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11 **HIGHLIGHTS:**

- Operating strategy influenced microbial community development,
 - and the predominant metabolic product in food waste fermentation
- Chain elongation was stimulated at higher retention times
- Lactic acid was the dominant product at higher organic loads

BACKGROUND: Effective food waste (FW) management must provide 16 cost-efficient resource and energy recovery to enable a sustainable circular 17 bio-economy¹. Of particular interest is fermentation with anaerobic mixed 18 microbial cultures (MMC) to generate products, such as biogas, lactic acid, 19 ethanol, H₂, or carboxylic acids (CA) ranging from volatile fatty acids (VFA), 20 to medium chain carboxylic acids (6-8 carbon atoms, MCCA) via chain 21 elongation. Various factors dictate fermentation outcome and MMC 22 composition, e.g. the organic loading rate (OLR) and retention times^{2, 3}. 23

To improve chain elongation yields in FW fermentation, various reactor 24 configurations, e.g. two-stage or leach bed, and supplementation of 25 chemicals have been considered^{4, 5}. However, studies have shown MCCA 26 production from FW is possible in a single-stage stirred tank reactor (STR)⁶. 27 The current study aimed to improve our understanding of how operation of 28 a single-stage STR steers product outcome and the microbial community. 29 The effects of OLR and hydraulic retention time (HRT) were assessed 30 separately by operating three sets of duplicate semi-continuous STR over 31 three HRT. We compared operation at a baseline HRT of 8.5 days and OLR 32 of 12 gCOD $L^{-1} d^{-1}$ (LH/LO) to operation at a higher HRT of 10.5 days 33 (HH/LO) and a higher OLR of 20 gCOD L⁻¹ d⁻¹ (LH/HO). FW was collected 34 from a full-scale industrial anaerobic digestion (AD) plant (GENeco, UK) and 35 seeding cultures from in-house acidogenic fermentation reactors. 36

RESULTS & DISCUSSION: In previous experiments, we found that the collected FW from the AD plant varies slightly in composition, is high in biodegradable total COD (130 to 163 gCOD L⁻¹) and already contains ethanol (5 to 13 g L⁻¹) and lactic acid (around 20 g L⁻¹). The three sets of 41 operating conditions resulted in three different fermentation outcomes and

42 MMC enrichments (Figure 1).



Figure 1: Average net yields of liquid fermentation outputs (left) and MMC composition at the genus level (right, colour per phylum) for varying operating conditions (i.e. combinations of HRT and OLR).

At lower OLR, lactic acid was net consumed and the total CA vields were 47 similar, yet product distribution differed. In the LH/LO reactors, $55 \pm 14\%$ 48 of the CA was butyric acid, and the fermentation was enriched in lactic acid 49 bacteria and VFA-producers typical of primary acidogenic fermentation. At 50 longer HRT in the HH/LO system, over three times more caproic acid was 51 obtained (up to 6.2 g L⁻¹). The semi-continuous fermentation cycle showed 52 consecutive fermentation stages of acidogenic fermentation and chain 53 elongation in HH/LO, in line with earlier reports on chain elongation in FW⁶. 54 The net consumption of both ethanol and lactic acid, and the presence of 55 genera related to ethanol- and lactate-based chain elongation, e.g. 56 *Caproiciproducens, Clostridium sensu stricto 12* and *Olsenella* spp., indicate 57 chain elongation could occur with either or both electron donor. Thus, 58 operating at longer HRT stimulated chain elongation. The residual butyric 59 acid and ethanol concentrations in the effluent of HH/LO suggest the 60 process could be further optimised. 61

⁶² When operating at higher OLR (LH/HO) minimal CAs were detected and ⁶³ predominantly lactic acid accumulated ($32 \pm 5 \text{ gCOD L}^{-1}$). More than twice ⁶⁴ the amount of NaOH was required to correct pH compared to operations at ⁶⁵ lower OLR. This was reflected in a MMC enriched in lactic acid producing ⁶⁶ and acid-resistant *Lactobacillus* and *Aeriscardovia* spp.

CONCLUSION: Food waste, with high biodegradable COD content, can 67 serve as a feedstock for a range of platform chemicals. This can be achieved 68 in a simple STR setup by manipulation of the operating conditions. 69 Operating at a high OLR resulted in an organic overload, characterised by 70 acidogenic lactic acid accumulation, where an acid-resistant community 71 thrived. At lower OLR, an increased residence time allowed the community 72 73 to evolve to perform chain elongation via secondary fermentation after acidogenesis. These results improve our understanding of the underlying 74 metabolic pathways in food waste fermentation, and demonstrate the 75 potential of adapting current single-stage food waste AD systems to 76 produce other bio-chemicals by modifying HRT and OLR. 77

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Water Res 2020, 169, 115215.

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