# Maximising resource recovery from carbon- and nutrient-rich mycoprotein fermentation wastewater using anaerobic digestion


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*Keywords: wastewater, anaerobic digestion, resource recovery, environmental microbiology.*



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## Introduction

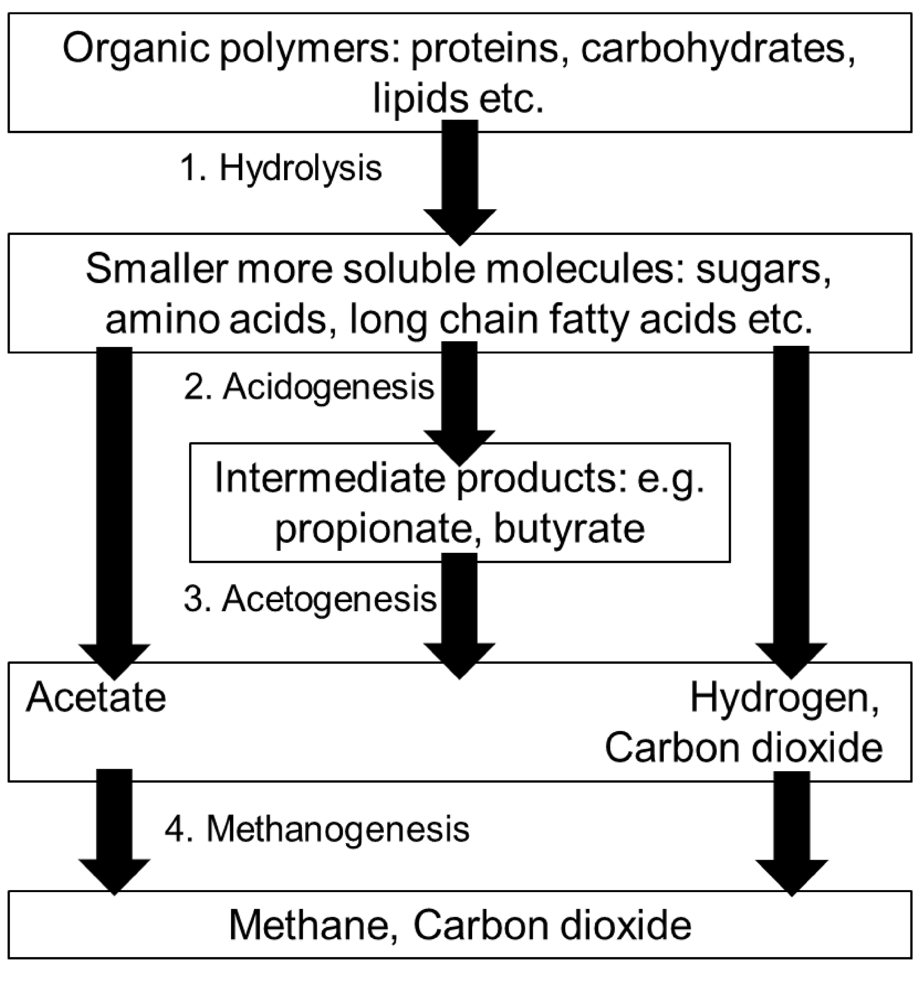


**Figure 1** | Overview of the four main stages of anaerobic digestion.

Anaerobic digestion (AD) offers an energy-efficient way to treat wastewater and recover organic carbon in the form of biogas (Figure 1). Food fermentation wastewater represents a carbon- and nutrient-rich waste stream with high resource recovery potential using AD. Despite known general characteristics such as chemical oxygen demand (COD), the specific chemical composition of complex food-fermentation wastewater remains largely undefined. Moreover, full scale AD plants tend to operate at suboptimal conditions to prevent process instability caused by disruptions to the microbiome at the metabolic level [1]. Previous research has suggested monitoring operational parameters in relation to microbiome structure and function for characterised substrate feeds [2,3].

**AIMS**

1) To conduct in-depth characterisation of Quorn wastewater as a representative of carbon- and nutrient-rich waste food-grade wastewater (Figure 2).

2) To address knowledge gaps on chemical characterisation which will allow further investigation of key trends of stable microbiomes underpinning AD.

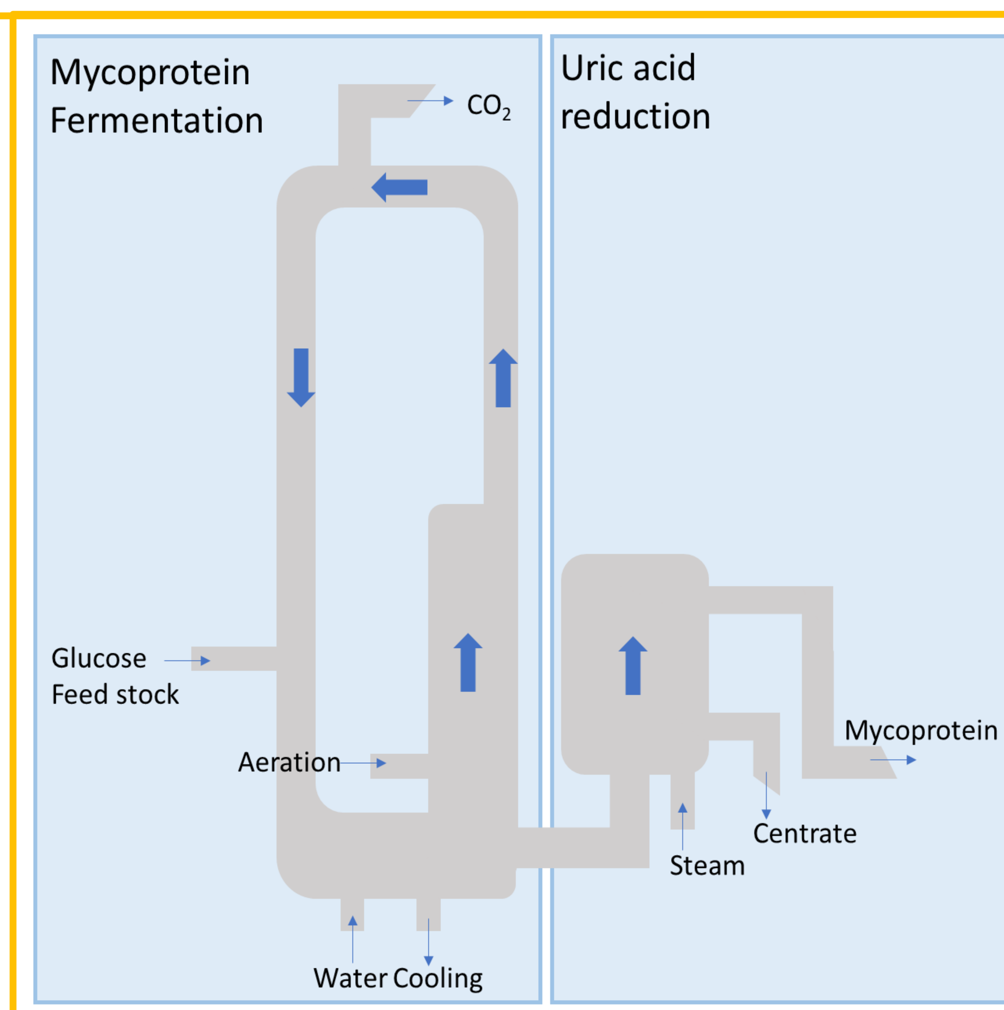


**Figure 2** | Mycoprotein fermentation process and centrate (wastewater) production.

## Results & Discussion

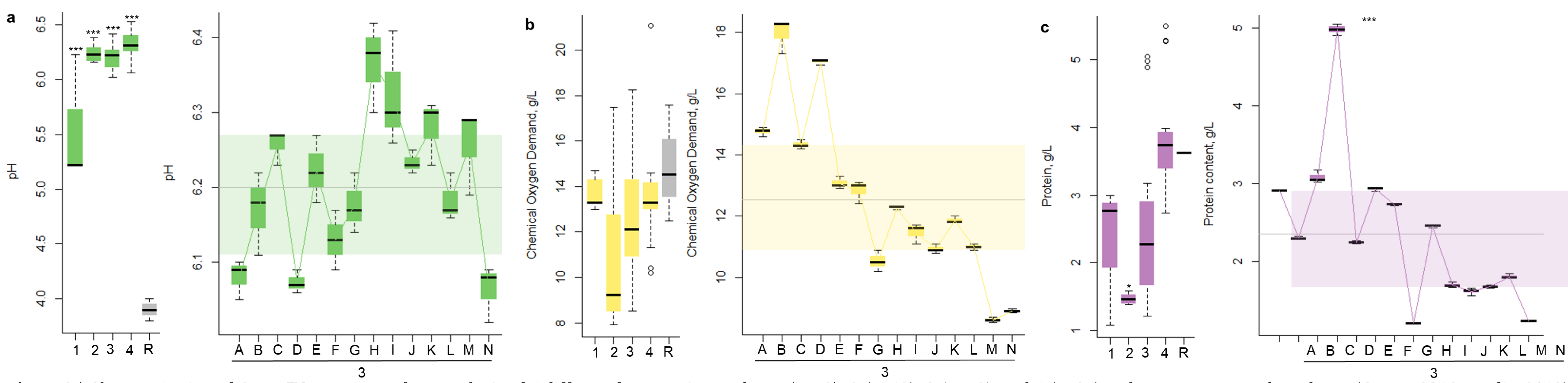


**Figure 3** | Characterisation of Quorn™ wastewater from analysis of 4 different fermentation cycles, 1 (n=13), 2 (n=13), 3 (n=42), and 4 (n=24) and previous reported results, R (Quorn, 2016, Veolia, 2013). A-N represent samples taken every 2 days over the course of a 30 day fermentation cycle (n=42). Results are presented as a box plot where the central line indicates the median, the box represents the interquartile range, the whiskers represent 1.5 times the interquartile range, and circles represent outlier values The mean average is shown as a straight grey line, and the interquartile range is highlighted. A one-way ANOVA and *post-hoc* Tukey's range test were performed with confidence intervals of 95% (*), 99% (**) and 99.9% (***). **a** pH; **b** Chemical oxygen demand (COD, g $O_2$/L); and **c** Protein content (g/L).

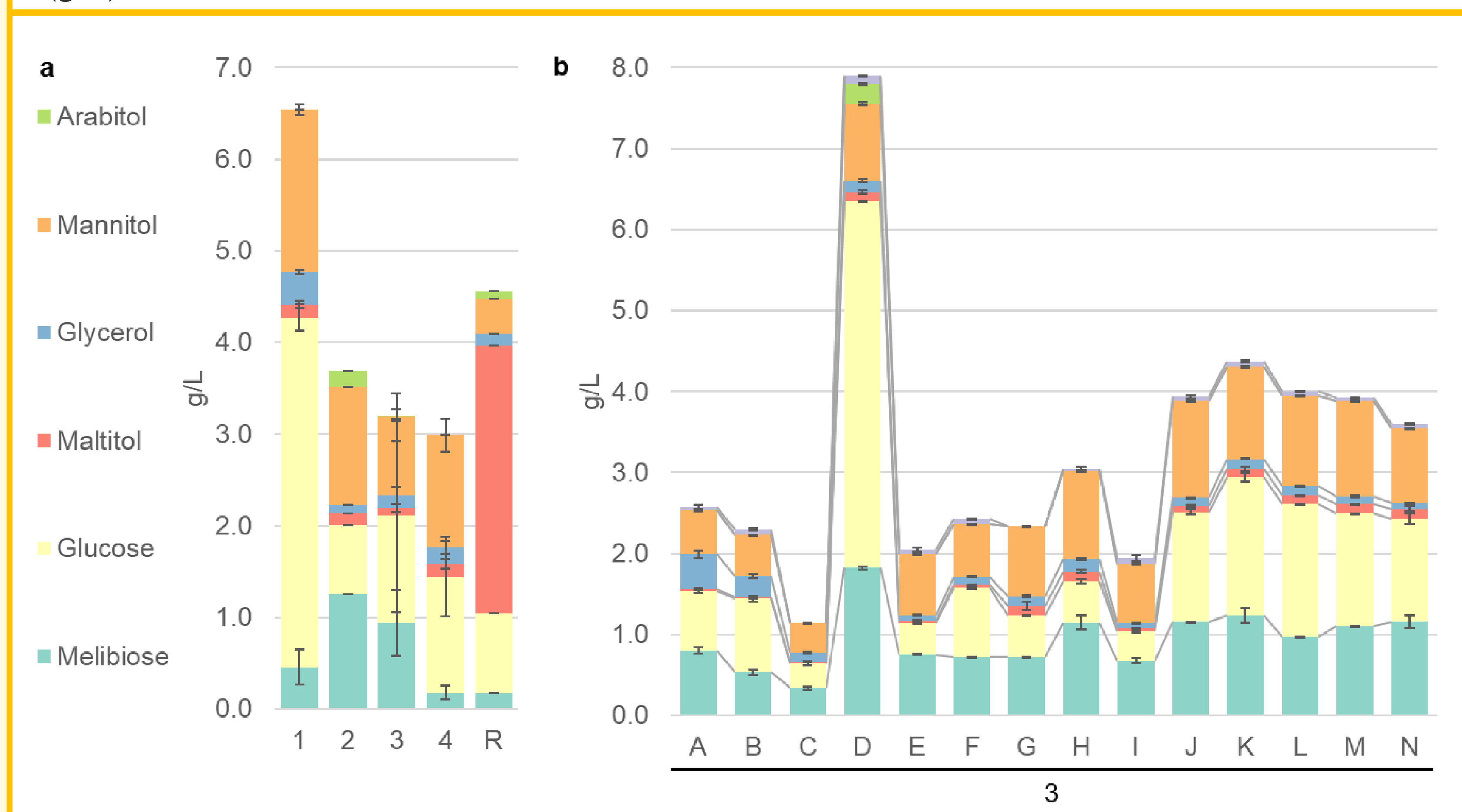


**Figure 4** | Total sugar and sugar alcohol content of mycoprotein fermentation wastewater samples analysed using high performance liquid chromatography. Error bars represent standard deviation. **a** Mean values of different fermentation cycles 1 (n=13), 2 (n=13) 3 (n=42), 4 (n=24) and previously reported results, R (Quorn, 2016); and **b** Time series analysis taken every two days over the course of a 30 day fermentation cycle (A-N)

We measured physicochemical parameters driving the AD process including: pH, COD, protein (as total nitrogen, Figure 3), nitrites, nitrates, ammonium, sulphate and phosphate.

We also measured soluble organic compounds in Quorn wastewater (including sugar and sugar alcohol content, Figure 4). Additionally, we assessed digestibility using biomethane potential assays, monitoring real time gas production and volatile fatty acid (VFA) content. Quorn wastewater from 4 different batches was characterised, and additional time series analysis were performed for batch 3 and 4 using samples taken over the course of a 30 day cycle (Figure 3, Figure 4).

**Results**

- pH was stable throughout the fermentation cycle (Figure 3) and was within range for direct application to AD systems without the need for pre-treatment or buffering.
- COD was shown to decrease over the fermentation cycle and values ranged from 8.53 to 18.28 g/L $O_2$, highlighting high organic matter content of Quorn wastewater, which would require treatment before release into the aquatic environment.
- Quorn fermentation wastewater has a complex sugar and sugar alcohol profile, representing high carbon recovery potential.
- Protein initially followed a similar trend to total sugar and sugar alcohol content, demonstrating high nutrient recovery potential.

## Conclusions and next steps

High carbon and nutrient recovery potential combined with near-neutral pH make Quorn wastewater an ideal candidate for resource recovery using AD.

In our future research we aim to investigate the structure and function of the microbiome underpinning AD of fully characterised Quorn wastewater in a continuous stirring treatment reactor (CSTR) configuration with a metagenomic and network analysis approach. We will also measure key physicochemical parameters of AD such as pH, COD and VFA production to investigate if microbiome structure and function can be decoupled from reactor stability.


**References: 1** Wu, Y. *et al.* Early warning indicators for mesophilic anaerobic digestion of corn stalk: a combined experimental and simulation approach. *Biotechnology for biofuels* **12**, 106 (2019). **2** Wu, D. *et al.* Anaerobic digestion: A review on process monitoring. *Renewable and Sustainable Energy Reviews* **103**, 1-12, doi:10.1016/j.rser.2018.12.039 (2019). **3** Demirel, B. & Scherer, P. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. *Reviews in Environmental Science and Bio/Technology* **7**, 173-190, doi:10.1007/s11157-008-9131-1 (2008).