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

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Anaerobic digestion (AD) offers an energy-efficient way to treat wastewater and recover organic carbon in the form of methane-containing biogas. Food fermentation wastewater represents a carbon- and nutrient-rich wastewater stream with high potential for resource recovery using AD. Despite known general wastewater characteristics such as the chemical oxygen demand (COD), the specific chemical composition of complex food-fermentation wastewater remains largely undefined. Moreover, full scale AD processes in industrial plants tend to operate at suboptimal conditions to prevent process instability caused by disruptions to the microbiome at the metabolic level (Wu et al., 2019b). Previous research suggested an interesting research direction would be to monitor operational parameters in relation to microbiome structure and function for characterised substrate feeds (Wu et al., 2019a, Demirel and Scherer, 2008). In our research, Quorn Foods was selected to represent advanced food fermentation technology; our study aims to address the knowledge gaps on chemical characterisation of carbon- and nutrient-rich Quorn fermentation wastewater, which will allow for further investigation of key trends of stable microbiomes underpinning AD.

In this study we not only measured the physicochemical parameters driving the AD process, including pH, COD, protein (as total nitrogen), nitrites, nitrates, ammonium, sulphate, and phosphate; but also the soluble organic compounds and nutrients in Quorn wastewater (for example protein, sugar, sugar alcohol, and amino acid contents). We have also assessed biodegradability using biomethane potential assays and monitoring real-time biogas production and volatile fatty acids (VFAs). Initial characterisation included fermentation wastewater samples taken from three different fermentation cycles and samples taken over the time course of a 30 day fermentation cycle. Using high performance liquid chromatography analysis, we found that Quorn fermentation wastewater has a complex sugar and sugar alcohol profile representing high carbon recovery potential (Figure 1). Protein appeared to initially follow a similar trend to total sugar and sugar alcohol content (Figure 1, Figure 2), demonstrating the nutrient-rich resource recovery potential of fermentation wastewater. We observed that pH was stable throughout the fermentation cycle and consistent with previous experimental values (Figure 2). We determined pH to be in the range for direct application to AD systems without the need for pre-treatment or buffering. COD values ranged from 8.53 to 18.28 g/L O_2, highlighting the high organic matter content of mycoprotein fermentation wastewater, which would require treatment before release into the aquatic environment. COD was shown to decrease over the fermentation cycle (Figure 2), but the overall mean average remained consistent with previously reported values (Figure 2).

Overall, the high carbon and nutrient recovery potential combined with a near-neutral pH make Quorn fermentation 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 fermentation wastewater in a continuous stirring treatment reactor (CSTR) configuration, using a patented Anaerotechnology auto-feed system), a metagenomic approach and network analysis. CSTR was chosen due to its simple operation and reported stability under stress conditions, such as overloading (Kim et al., 2002). We will also monitor 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.

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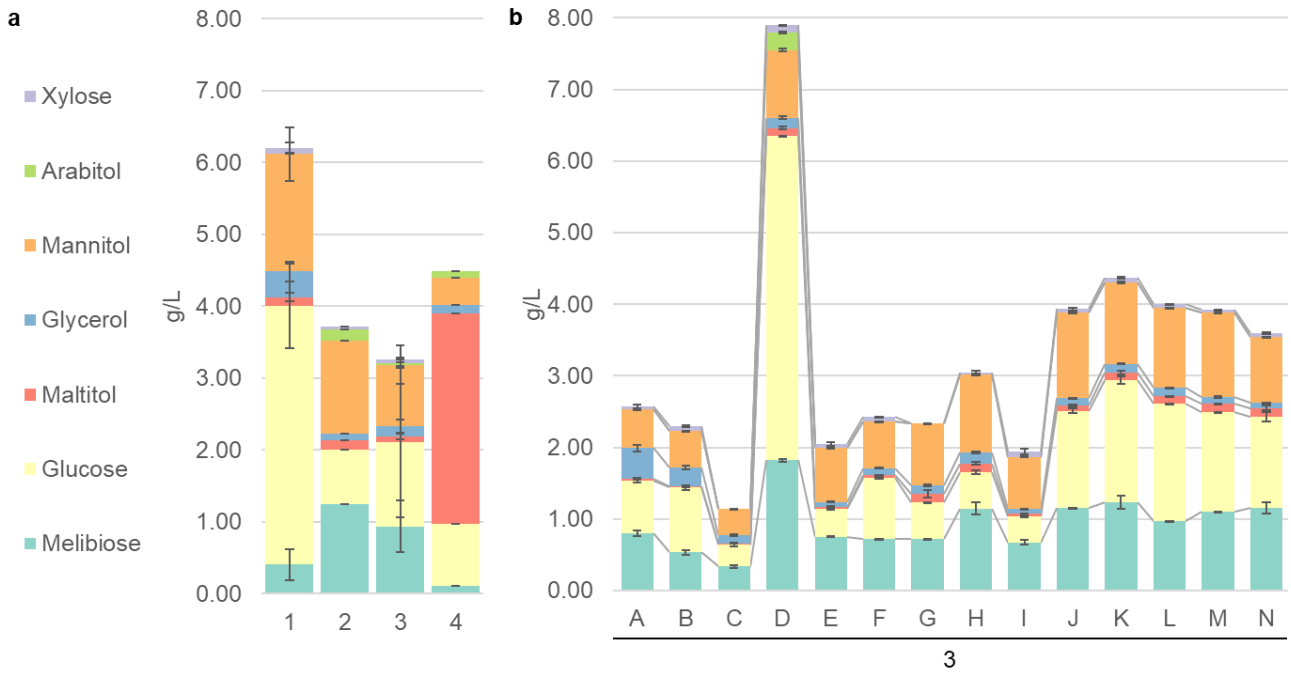

Figure 1 | Total sugar and sugar alcohol content of mycoprotein fermentation wastewater samples analysed using high performance liquid chromatography for arabitol, mannitol, glycerol, maltitol, glucose and melibiose. All measurements were carried out in triplicate and measured in g/L. Error bars represent the standard deviation. **a** The mean values for the different fermentation cycles 1 (n=13), 2 (n=13) 3 (n=42) and previously reported results, 4 (Quorn, 2016); and **b** Detailed analysis of 14 different samples (A-N) taken every two days over the course of the 3rd 30 day fermentation cycle.

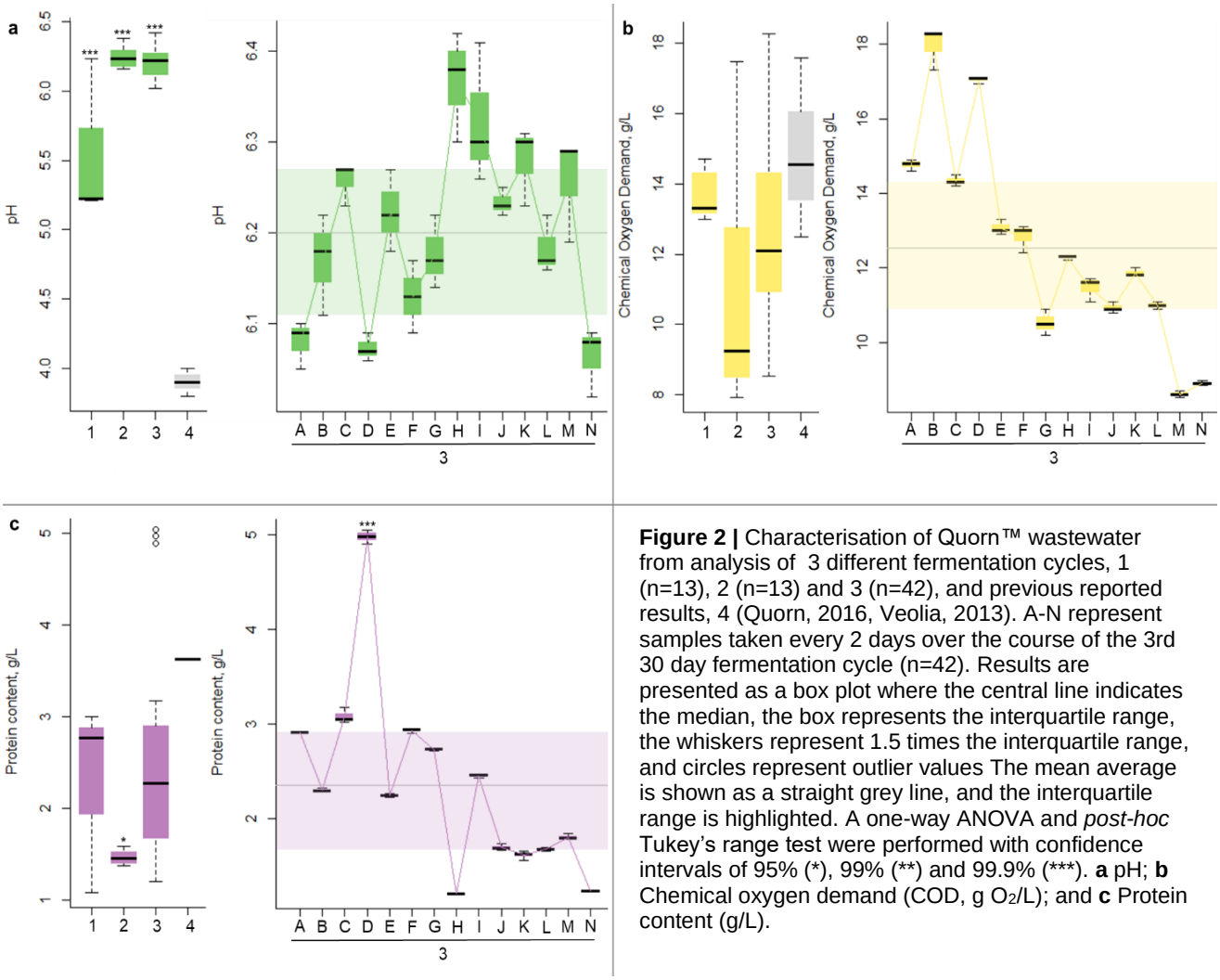

Figure 2 | Characterisation of Quorn™ wastewater from analysis of 3 different fermentation cycles, 1 (n=13), 2 (n=13) and 3 (n=42), and previous reported results, 4 (Quorn, 2016, Veolia, 2013). A-N represent samples taken every 2 days over the course of the 3rd 30 day fermentation cycle (n=42). Results 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).