Technologies for recycling waste agricultural biomass

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1. Aim

Food security is a global challenge and waste plant biomass is an environmental problem. However, biomass could be recycled into a nutritious substrate for farmed insects. As a natural component of the diet of farmed fish, chicken and pigs, fly larvae have the potential to help meet the demand for protein in animal feed (Figure 1).

Fig 1. Black soldier fly reared for farmed animal feed.[1]

Lignocellulosic plant biomass is composed of the sugar polymers cellulose and hemicellulose, and the polymer lignin. Some plant waste, such as palm waste, is tough and needs to be broken down using microbes (anaerobic digestion), providing a substrate for fly larvae and producing methane gas, used as a sustainable energy source. We aimed to optimise conditions for recycling this waste using analytical chemistry techniques and multivariate statistics (known as "metabolomics").

2. Experimental Methods

A schematic of the process is shown in Figure 2. Analytical chemistry techniques (nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography high resolution mass spectrometry (LC-HRMS)) were used to analyse palm empty fruit bunches (EFB) after various pre-processing methods to determine their biological composition. This was repeated after anaerobic digestion (AD) to see how different AD conditions changed the biological composition of the EFB. Biogas production was also measured during AD. Confirmatory analysis experiments[2] were performed to identify the biological components causing differences in composition between waste biomass preparation and AD digestate conditions (e.g. sugars or fungal toxins). This was achieved by comparing results of the investigation with results obtained from corresponding analytical standard chemicals of the suspected component.

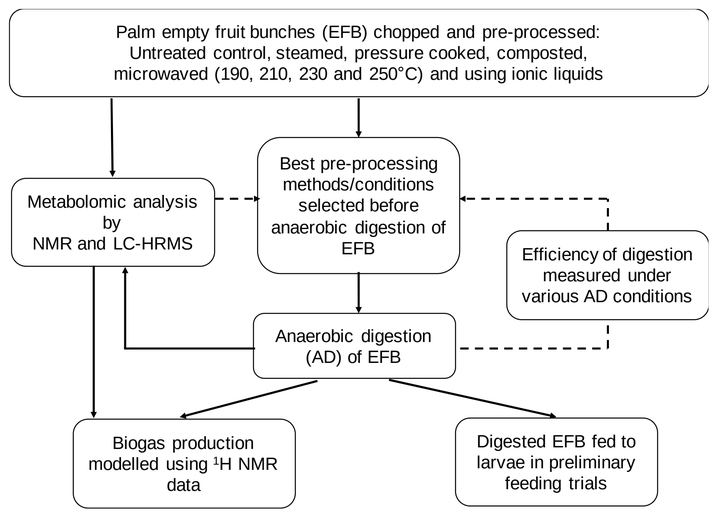

Fig 2. Flow diagram showing the procedure followed for optimising the breakdown of palm EFB and the production of biogas and substrate for insect rearing.

3. Results

Pre-processing of EFB (before AD):

Statistical pattern recognition on the analytical chemistry data (Figure 3) showed that compositional differences existed between palm waste samples when they were pre-processed in different ways. The clustering/spread of the samples on the principal component analysis (PCA) scores plots in Figure 3 show the similarities or differences between pre-processed samples. The biological components that changed between samples were identified using analytical standard chemicals, and are shown in Table 1.

Biological components ("metabolites") identified	
Vanillin	Syringaldehyde
Glucose	Levoglucosan
Xylose	Fusaric Acid
Malic Acid	Disaccharides

Table 1. Confirmed metabolites in pre-processed EFB, identified using the corresponding analytical standards.

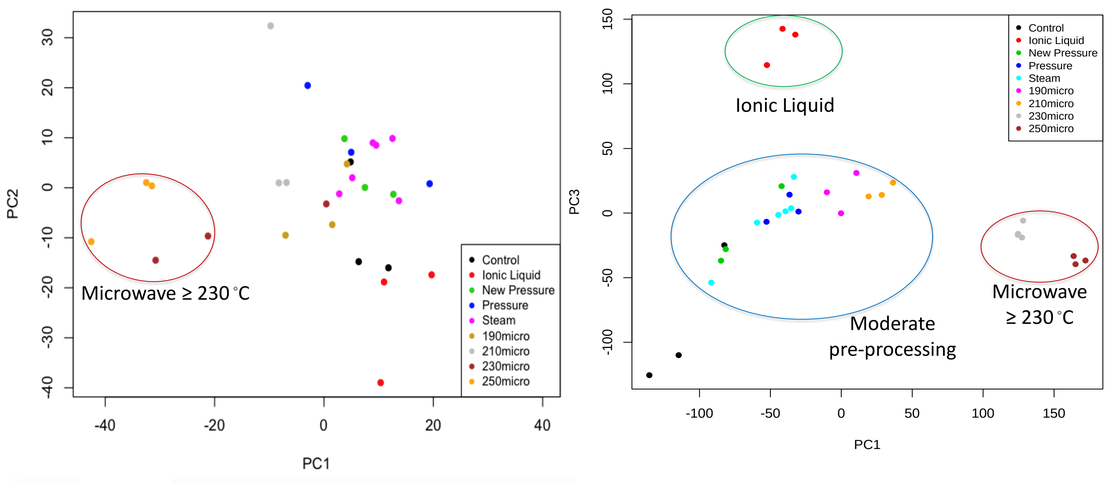

Fig 3. PCA scores plots of LC-MS data (left) and NMR data (right) of pre-processed EFB before anaerobic digestion.

Samples microwaved at high temperature seemed to be most different to other pre-processed samples. Many flavours and fragrances (e.g. vanillin), sugars such as glucose and xylose, and shorter chains of lignin (indicating a greater breakdown of the long lignin polymer) were found to be in higher concentration in EFB samples microwaved ≥230°C. The sugar levoglucosan, a "burnt biomass marker", was also identified in these samples. It has previously been shown[3] that this product can affect microorganism growth therefore it was possible that AD experiments may be affected by its presence. It was concerning that the fungal toxin fusaric acid was also detected, in case of toxicity to the larvae or the possibility that it may enter the food chain. Longer chains of lignin were higher in concentration in moderate pre-processing such as steaming (indicating less lignin breakdown) than when microwaving ≥230°C.

Ionic liquids (IL) are liquid salts and are increasingly being used to break down waste biomass[4]. Samples pre-processed by IL resulted in a higher concentration of disaccharide sugars, however, trace IL was detected in the EFB sample after pre-processing, and it was unknown if this would affect the subsequent AD experiments or be toxic to the larvae during feeding trials.

Digested EFB (after AD):

PCA of data from analysis of digested EFB (after anaerobic digestion) showed that compositional differences existed between samples again, based on original pre-processing methods (Figure 4). Most of this variance was again from the breakdown of lignin. Short chain lignins were in higher concentration for EFB samples that were microwaved ≥230°C before AD, moderate pre-processing before AD left longer chain lignins after AD, and acidic pH during AD improved lignin breakdown.

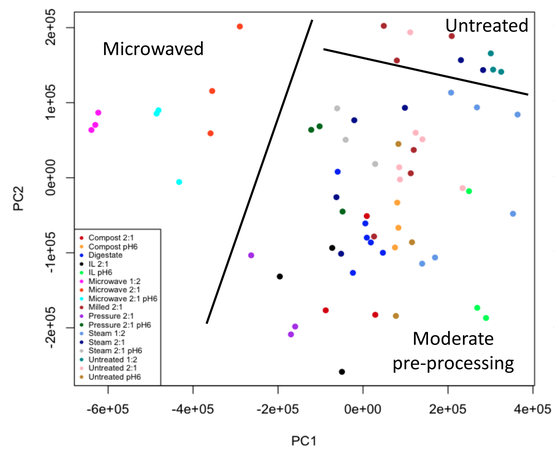

Fig 4. PCA scores plot of NMR data of palm EFB after anaerobic digestion

Levoglucosan produced when microwaving inhibited the growth of some microorganisms during AD, confirmed by the lower levels of biogas produced for experiments where a higher EFB ratio to digestate inoculum was used. The fungal toxin fusaric acid was not detected post-AD, but trace IL was still present. However, IL was not toxic to larvae (Figure 5A), and actually produced the most promising larvae substrate from the palm EFB, as well as the most biogas (Figure 5B).

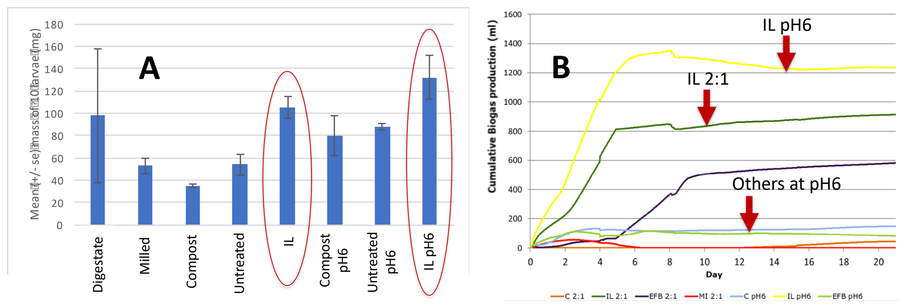

Fig 5. (A) Results of preliminary feeding trial with EFB after 4 days digestion; (B) plots of cumulative biogas production during AD, under different pre-processing / experimental conditions.

<u>Conclusion</u>: Optimal conditions for larvae substrate and biogas production are to pre-process palm EFB by IL, followed by AD at pH6 with 2:1 digestate:EFB ratio.

We would like to thank the EPSRC and The Douglas Bomford Trust for funding this research.

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