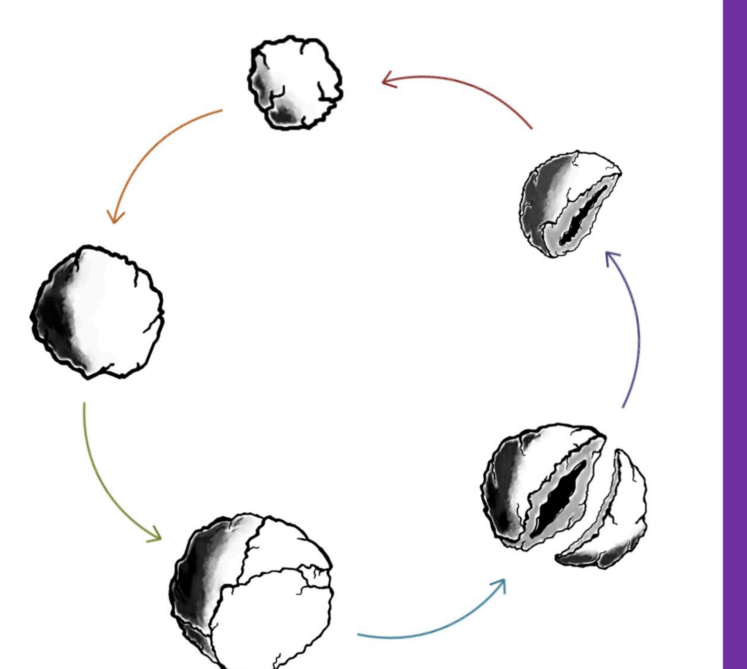
Size Matters For Structure And Function Of Anaerobic Sludge Granules, Supporting A Biofilm Life-Cycle Model

*Anna Trego**, C. Morabito*, S. Mills*, S. Connelly**, I. Bourven***, G. Guibaud***, C. Quince****, U. Zeeshan Ijaz** & G. Collins*,**

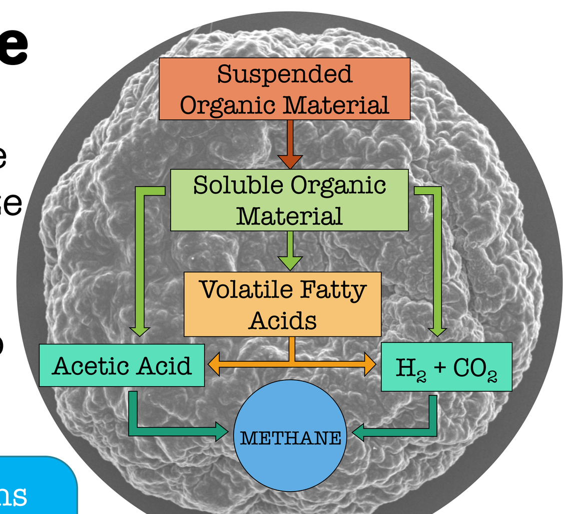
NUI Galway
OÉ Gaillimh

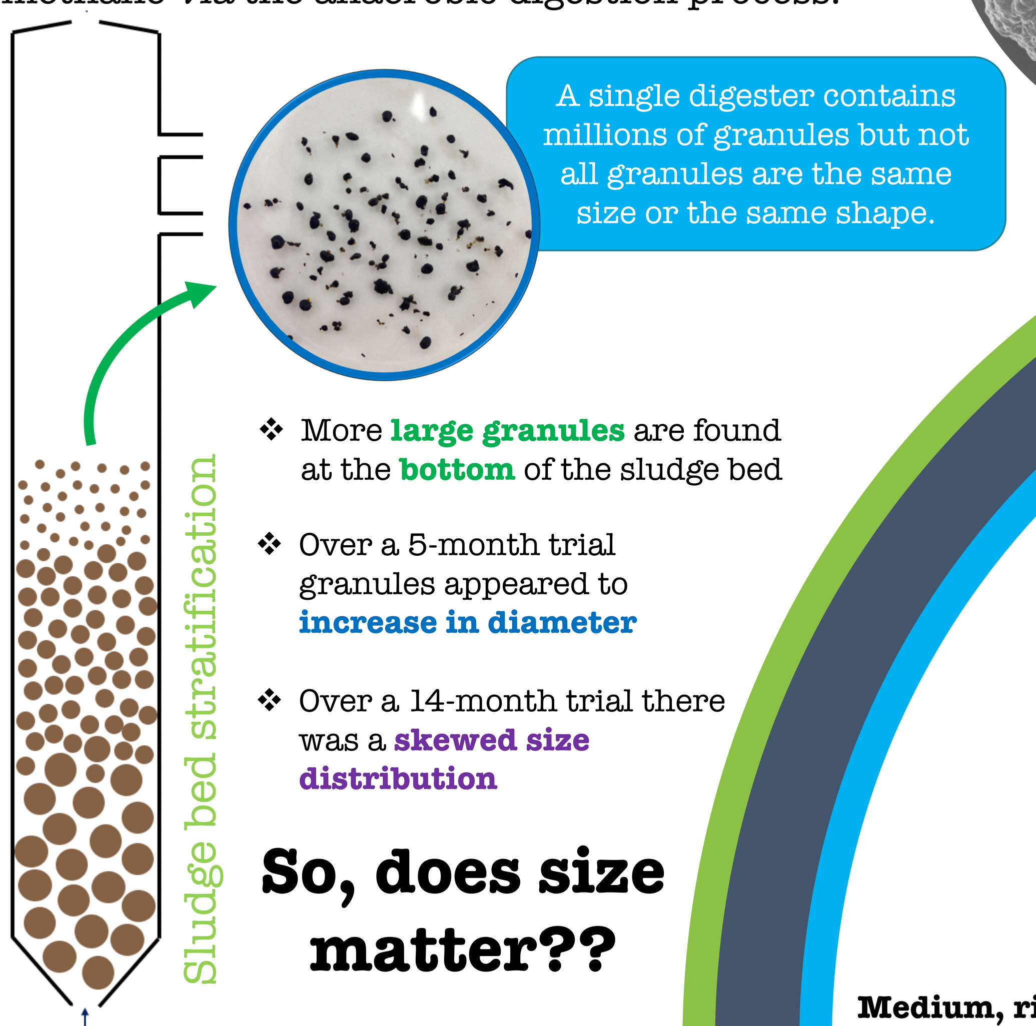
* School of Natural Sciences, National University of Ireland Galway, University Road, Galway, Ireland (E-mail: *a.trego1@nuigalway.ie*)
** School of Engineering, The University of Glasgow, Oakfield Avenue, Glasgow G128LT
*** GRESE, Faculté des Sciences et Techniques, Université de Limoges, 123 Avenue A. Thomas, 87060 Limoges Cedex, France
****Warwick Medical School, University of Warwick, Coventry CV4 7AL, United Kingdom



Not all granules are the same

Anaerobic granules are **small**, usually **spherical**, biofilm aggregates that form spontaneously inside up-flow anaerobic digesters. Granules range in size from 0.1 to 5 mm in diameter and contain the **entire community necessary** for the complete mineralization of suspended organic material into methane *via* the anaerobic digestion process.





- More **large granules** are found at the **bottom** of the sludge bed
- Over a 5-month trial granules appeared to **increase in diameter**
- Over a 14-month trial there was a **skewed size distribution**

So, does size matter??

Granules differ in structure and function

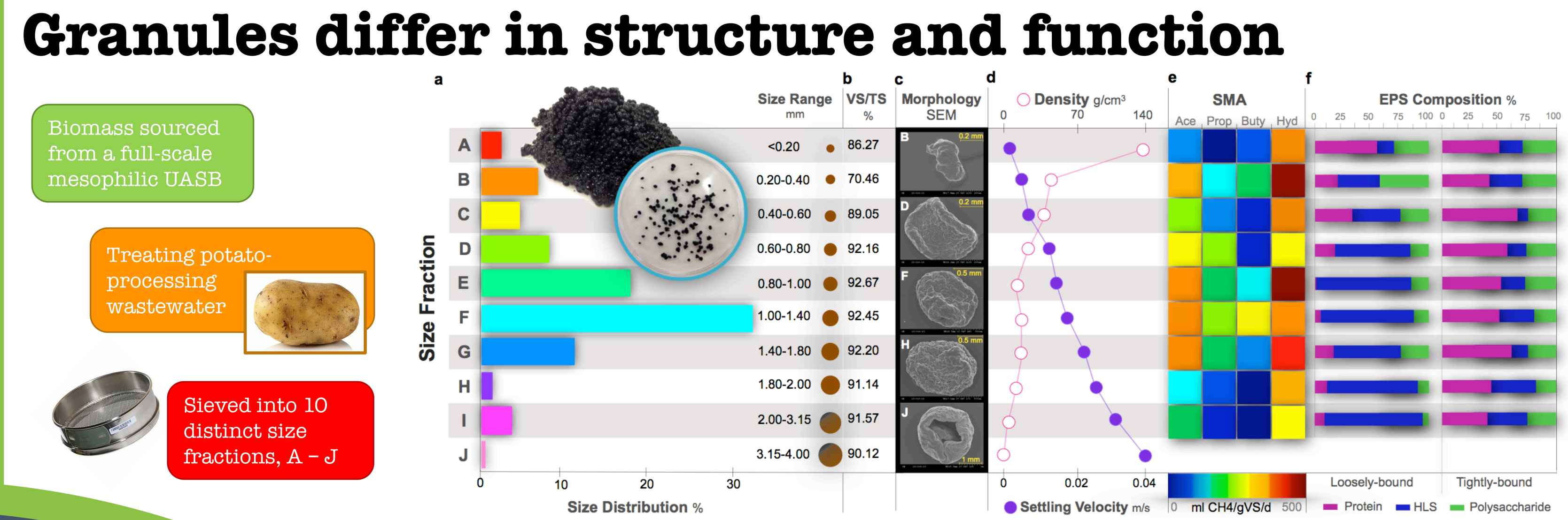
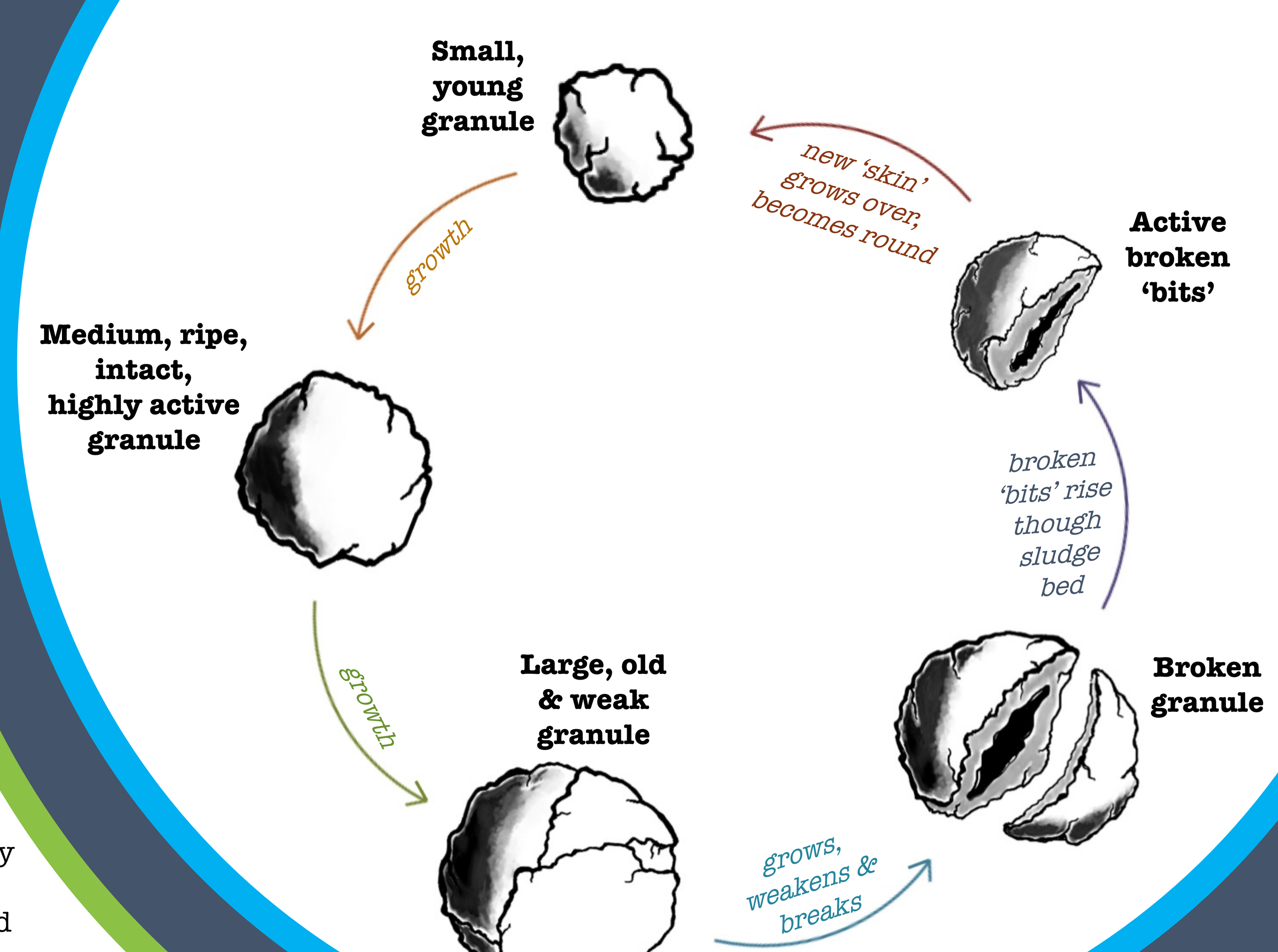


Figure 1. Physico-chemical and physiological data from granule size fractions, A – J. **(a)** Bar plot indicating the size ranges of respective fractions, along with relative volumetric contributions to the sludge; **(b)** VS proportions of TS; **(c)** typical scanning electron microscopy (SEM) micrographs of selected granules (from fractions B, D, F, H and J); **(d)** scatter plot illustrating density, and settling velocity, of granules (*n*=10) from each size fraction; **(e)** heat map depicting specific methanogenic activity (SMA) of sludge samples (*n*=3) from each size fraction (except fraction J) against acetate (Ace), propionate (Prop), butyrate (Buty) and H_2/CO_2 (Hyd); and **(f)** stacked bar charts showing relative concentrations of proteins, humic-like substances (HLS) and polysaccharides components in loosely-bound and tightly-bound-EPS extracted from each size fraction.

The Granular Growth Hypothesis:

A Life Cycle Model for Anaerobic Granules



Differences in diversity explained by a subgroup of determinant OTUs

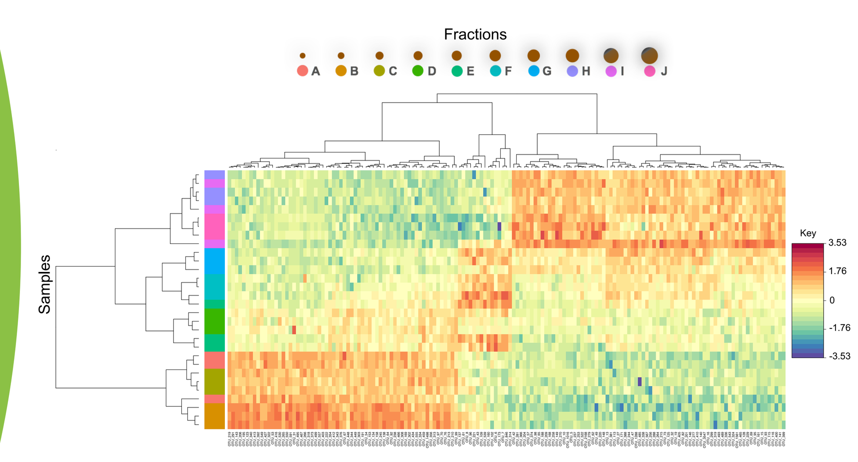


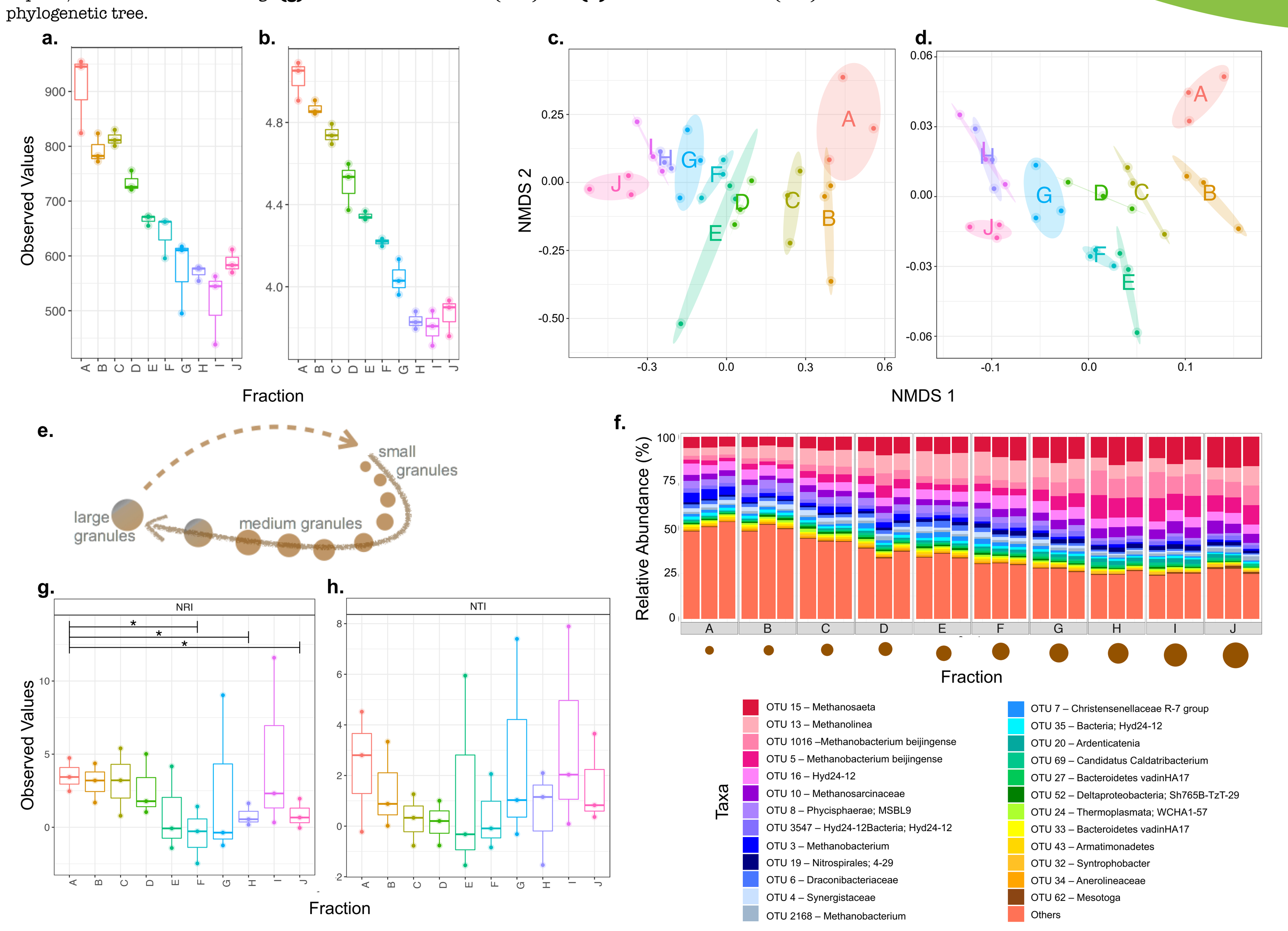
Figure 3. Heat map showing log ratios of abundance of the 155 determinant OTUs from sPLS analysis across the ten size fractions (A-J; in triplicate, *n*=30) ordered using UPGMA clustering and showing community similarity (y-axis) and the distribution of OTUs (x-axis).

One hundred and fifty-five OTUs from across the ten size fractions were determined to be the **determinant OTUs, contributing to changes in the community structure** across the sizes. There are three distinct groupings of these OTUs according to abundance and across the sizes.

Granules display diversity gradients across sizes

Alpha diversity analysis indicated a **strong diversity gradient** across the size fractions with a significantly higher rarefied richness (Fig 2a) and Shannon entropy (Fig 2b) in the smaller granules than in the larger granules. Additionally, beta diversity analysis revealed a **highly significant differentiation pattern** Fig 2c-d.

Figure 2. Microbial diversity, and community structure, in samples (*n*=3) from across each of the ten size fractions, A-J, according to variances in the 16S rRNA gene. *Alpha diversity*: box plot of the **(a)** rarefied species richness and **(b)** Shannon Entropy. *Beta diversity*: Non-Metric Multidimensional Scaling (NMDS) using **(c)** Bray-Curtis dissimilarity and **(d)** weighted UniFrac distances, where each point corresponds to the community structure of one sample, size fractions are indicated by colour, and the ellipses are drawn at a 95% CI; **(e)** depiction of the proposed granule growth, or life-cycle, trajectory; **(f)** community structure based on relative abundance of the top-25 most abundant OTUs from across each size fraction, where 'others' refers to all OTUs not included in the 'top-25'; *Environmental Filtering:* **(g)** Net Relatedness Index (NRI) and **(h)** Nearest Taxa Index (NTI) from the phylogenetic tree.



Determinant OTUs correlate significantly with physico-chemical data

Loosely bound extracellular polymeric substances (LB-EPS) correlate significantly with small and large granules

Medium-size granules have **significantly positive correlations** with specific methanogenic activity assays (SMA)

Medium-sized granules appear to sit within a **'transition zone'** where correlations begin to shift from positive to negative

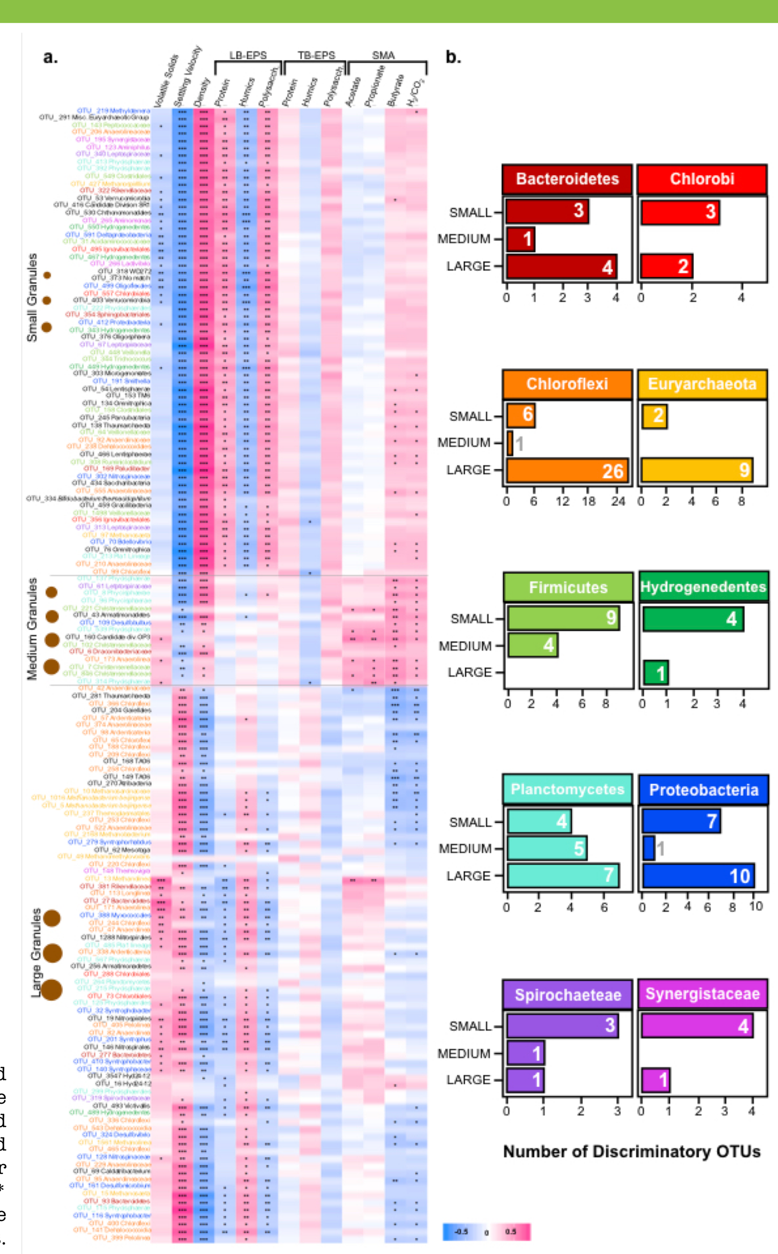
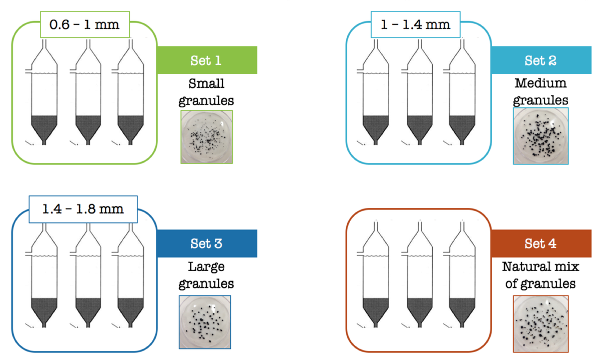


Figure 4. **(a)** Heatmap depicting the 155 significantly determinant OTUs coloured according to taxonomy (except where black), from sPLS analysis across the ten size fractions binned into three size groups: small (fractions A-C), medium (fractions D-G) and large (fractions H-J), and showing correlations with physico-chemical variables calculated using the Kendall rank correlation coefficient, where significant positive (pink) or negative (blue) correlations are marked with * (*Adj. P* < 0.05), ** (*Adj. P* < 0.01) or *** (*Adj. P* < 0.001). **(b)** Number of determinant OTUs (x-axis) from major phyla that were found in small, medium, and large bins.

Granules are not static entities inside anaerobic digesters

Twelve identical lab-scale (2L) EGSB reactors were operated in four sets of triplicates: the first set (R_{S1}–R_{S3}) containing only small (S) granules; the second set (R_{M1}–R_{M3}) containing only medium (M) sized granules; the third set (R_{L1}–R_{L3}) containing only large (L) granules; and the fourth set (R_{N1}–R_{N3}) was inoculated with the unfractionated (naturally distributed) sludge (N).



Reactors were operated identically for 51 days

On day 51 (D51) of the trial, sludge was removed for fractionation into XS, S, M, L, and XL groups.

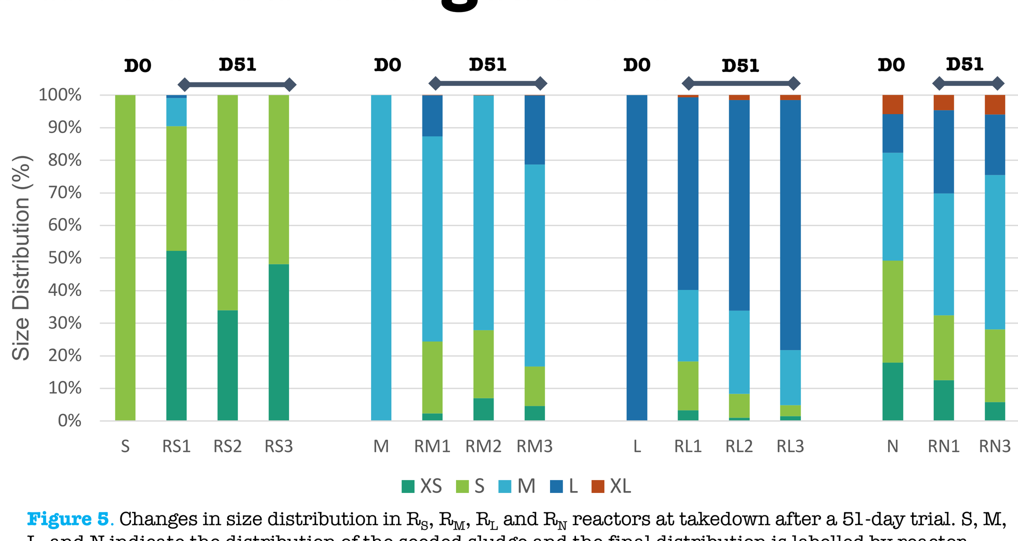


Figure 5. Changes in size distribution in R_S, R_M, R_L and R_N reactors at takedown after a 51-day trial. S, M, L, and N indicate the distribution of the seeded sludge and the final distribution is labelled by reactor.

Acknowledgements

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