Profiling soluble organic content in sewage sludge fed to anaerobic digester by NMR spectroscopy: a Danish WWTP survey

gd@bio.aau.dk

Giulia Dottorini, Florian-Alexander Herbst, Marta Nierychlo, Reinhard Wimmer, Per Halkjær Nielsen

Center for Microbial Communities, Aalborg University, Aalborg Denmark

Introduction

Anaerobic Digestion (AD) is a widely spread technology crucial for energy production (biogas) from organic wastes, nutrients recovery (P, N) and stabilization of sewage sludge produced along Wastewater Treatment Plants (WWTPs). Sewage sludge is a valuable feed for AD due to its high organic content. In Aalborg West WWTP (Denmark), sewage sludge fraction fed into digester includes mainly primary sludge and its mixture with surplus sludge coming from the secondary settler.

How does the feed composition of anaerobic digesters look like?
Previous studies (e.g. Satpathy et al. 2016; Kirkegaard et al. 2017) pointed out the importance of feed stream compositions in shaping microbial community structure involved in AD, influencing the digester's performance. A comprehensive profiling of organic compounds in sewage sludge before its subsequent feeding to AD is still lacking and it is needed to better understand and optimize biochemical dynamics of AD.

How to get highly detailed information about digester's feed profile?
Beside the traditional approaches (COD or HPLC measurements) to characterize organic matter composition, Nuclear Magnetic Resonance (NMR) spectroscopy represents one of the most powerful analytical tools to unravel molecular details, even in complex matrices such as sludge samples and wastewater (Alves Filho et al. 2015).

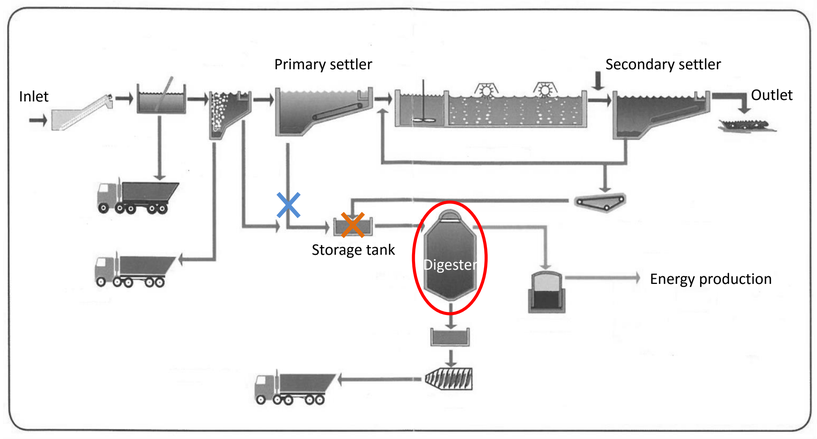
Aim

Develop and optimize a NMR spectroscopy method to characterize the composition of the soluble fraction of primary sludge at Aalborg West WWTP used to feed the anaerobic digester.

Methods

Digester's feed samples:

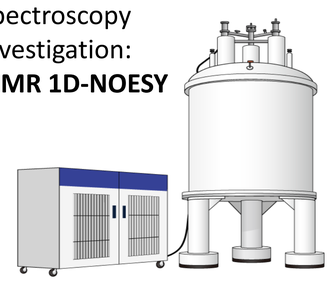
- ✓ primary sludge
- ✓ primary sludge + surplus sludge



Aalborg West WWTP and sampling points of this survey.

Soluble fraction preparation

Spectroscopy investigation: **^{1}H-NMR 1D-NOESY**



BRUKER AVIII - 600 MHz NMR spectrometer

Signal identification and quantification: **ChenomX NMR software**

Moreover:

Methods comparison:
- ✓ *COD and HPLC analysis*

COD kit LCK 414 5-60 mg/l O_2

HPIC Dionex ICS-5000 system

Microbial communities composition investigation:
- ✓ 16S rRNA gene amplicon sequencing

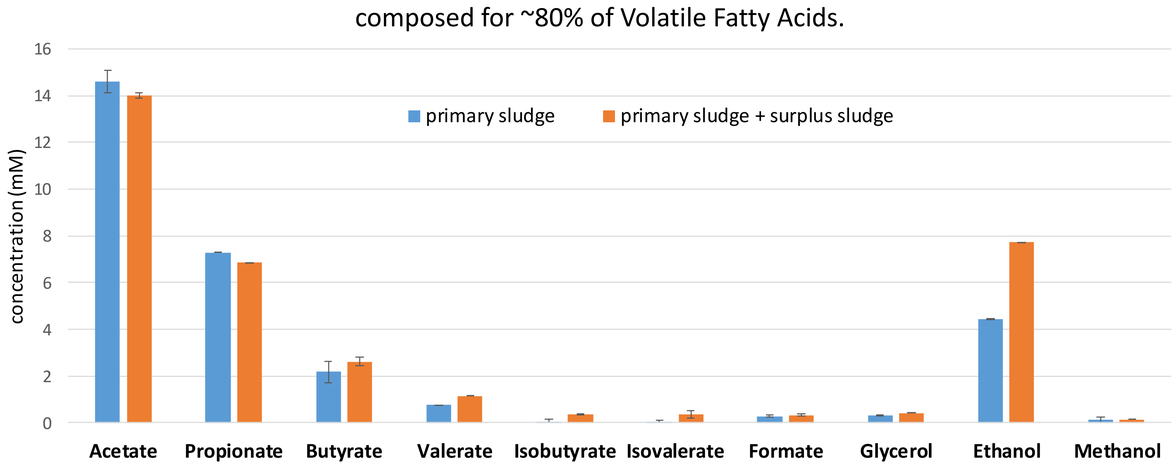
Illunina MiSeq

Conclusions

Method optimization allowed to explain up to 90% of the soluble COD profile in the anaerobic digester feed applying an unbiased and untargeted NMR approach. The results imply that, in Aalborg West WWTP, the digester's feed organic profile is determined mainly by primary sludge composition, rather than by surplus sludge: volatile fatty acids feeding the digesters represent ~80% of sludge soluble fraction. Instead, microbial community composition introduced into the digester is influenced by both feeds.

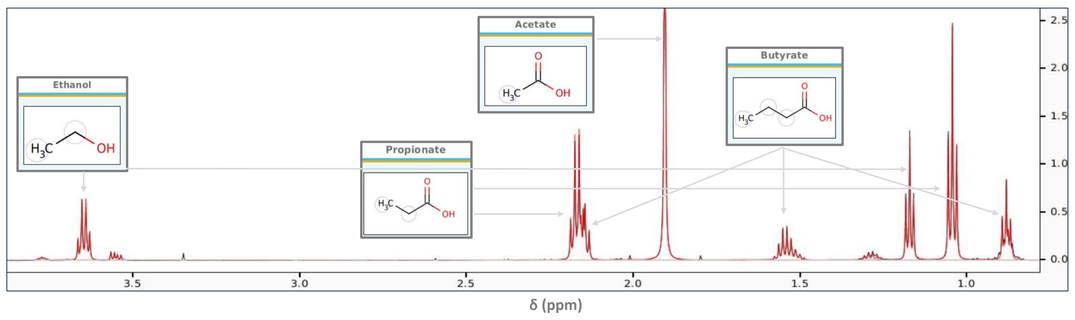
Results

The soluble fraction of digester's feeds rely on the organic profile of primary sludge, composed for ~80% of Volatile Fatty Acids.



Composition and concentration of compounds detected by H^{1}-NMR.

Most of the measured signals (black) can be explained after *in silico* analysis (red).



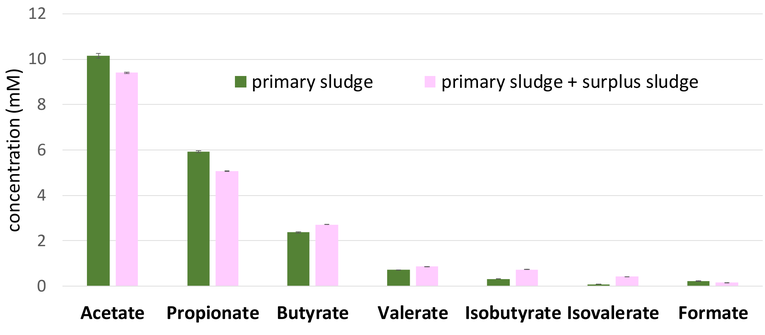
^{1}H-NMR spectrum of primary sludge.

Up to ~90% of the soluble COD can be explained by the compounds quantified by NMR.

	COD (g/l)		
	ThOD (NMR)	**COD kit**	**estimate (TSS)**
	supernatant	**supernatant**	**raw sludge**
primary sludge	3.1	3.5	66.9
primary sludge + surplus sludge	3.9	4.2	64.6

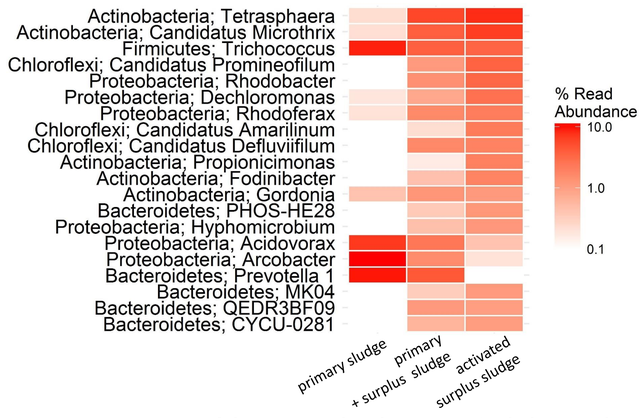
Chemical Oxygen Demand (COD) characterization of different digester's feeds fractions: in supernatant, it was determined by COD kit and by Theoretical Oxygen Demand (ThOD) based on the concentrations detected by NMR; in raw sludge, it was estimated based on Total Suspended Solid (TSS) measurements.

Targeted analysis of VFAs in digester's feeds confirms the accuracy of NMR approach.



Composition and concentration of Volatile Fatty Acids (VFAs) detected by HPIC.

Microbial community composition of digester's feed is influenced by the composition of primary sludge as well as surplus sludge.



Heatmap of the 20 most abundant genera in sludge samples detected by 16S rRNA gene amplicon sequencing. Activated surplus sludge sample is included for comparison.