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# Anaerobic Digestion Conference AD16

## Accelerating natural cycles

23-27 June 2019, Delft, The Netherlands



**16<sup>TH</sup>**  
**IWA World Conference**  
**on Anaerobic Digestion**  
Accelerating natural cycles with anaerobic digestion

**IWA**  
the international  
water association

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## Wednesday 26-06-2019

Time	Session 1	Session 2	Session 3
	Plenary session Chair: D. Batstone & M.K. de Kreuk Auditorium		
9.00-9.30	Keynote presentation <b>Microbial Ecology for Resource Recovery with Anaerobic Digestion</b> Mark van Loosdrecht, Technical University of Delft		
9.30-10.00	Keynote presentation <b>Solid state anaerobic digestion of mixed organic waste</b> Elizabeth Edwards, University of Toronto		
10.00-10.30	Coffee break + posters		
	<b>Modelling and Control</b> Chair: R. Kleerebezem & S. Astals-Garcia  Auditorium	<b>Resource Recovery /Bio-polymer production using anaerobic systems</b> Chair: J.A. Field  College room A	<b>Sludge and slurry digestion</b> Chair: L. Borzacconi & X. Flotats  College room D
10.30-10.50	<b>A revision of microbial growth yields in anaerobic digestion</b>  J. Rodríguez, M. Paton, United Arab Emirates	<b>Electroactive methanogens</b>  A.-E. Rotaru, Denmark	<b>Co-digestion and pretreatment improve microalgae anaerobic digestion</b>  I. Ferrer, M. Solé-Bundó, M. Garfí, Spain
10.50-11.05	<b>Demand-driven biogas production by substrate management – Investigations in process stability at different scales</b>  E. Mauky, S. Weinrich, H.J. Naegele, H.F. Jacobi, J. Liebetrau, M. Nelles, Germany	<b>Integrating recovery of nutrients and energy: An economic assessment of fifteen scenarios for pig manure treatment</b>  J. de Vrieze, G. Colica, C. Pintucci, C. Pedizzi, M. Spiller, M. Carballa, S.E. Vlaeminck, Belgium	<b>High solids anaerobic digestion of lignocellulosic biomass via oxidation-reduction potential-based micro-aeration</b>  S.K. Khanal, D. Nguyen, Z. Wu, S. Shrestha, P.H. Lee, L. Raskin, United States
11.05-11.20	<b>Predictive models of AD inhibition can be built by integrating independent studies</b>  O. Chapleur, S. Poirier, S. Déjean, K. Lê Cao, France	<b>Chain oddity: understanding odd-chain elongation and why it behaves in an odd way</b>  R. Ganigué, P. Candy, C. Petrognani, B. Ulčar, K. Rabaey, Belgium	<b>Continuous stirred tank reactors in series: an approach to enhance the enzymatic hydrolysis and sludge reduction in anaerobic waste activated sludge digestion?</b>  H. Guo, R. Nair, F. Tonin, A. Hendriks, J.B. van Lier, M. de Kreuk, The Netherlands

	<p><i>Modelling and Control</i> Chair: J. Rodriguez &amp; J. Ferrer</p> <p>Auditorium</p>	<p><i>Resource Recovery /Bio-polymer production using anaerobic systems</i> Chair: J. de Vrieze &amp; G. Buitrón</p> <p>College room A</p>	<p><i>Sludge and slurry digestion</i> Chair: C. Holliger &amp; I. Ferrer</p> <p>College room D</p>
14.00-14.15	<p><b>Anaerobic sequential batch reactors for high-efficiency treatment of slow degradable industrial wastewater</b></p> <p>S.P.R. Pacheco-Ruiz, A.M. Ferreira, The Netherlands</p>	<p><b>Nitrogen recovery from anaerobically digested blackwaters using Bioelectrochemical systems</b></p> <p>E. Borràs, D. Molognoni, M. Aliaguilla, P. Bosch-Jimenez, M. P. Bernicola, J. García-Montaño, S. Sanchis, Spain</p>	<p><b>Dry anaerobic digestion of sewage sludge in small and medium WWTP</b></p> <p>J. Garcia-Aguirre, E. Aymerich, M. Esteban-Gutierrez, L. Pastor, J.E. Sánchez-Ramírez, S. Doñate, R. Romaguera, C. Lardin, E. Mino, Spain</p>
14.15-14.30	<p><b>Insights into the synergistic effects of the anaerobic co-digestion of sludge and food waste through the modelization of semi-continuous experimental tests</b></p> <p>D. Montecchio, P. Pagliaccia, V. Di Castro, A. Gallipoli, A. Gianico, S. Rossetti, B. Tonanzi, C. Braguglia, Italy</p>	<p><b>Evaluation of protein composition influence on yields and selectivity of volatile fatty acids production</b></p> <p>R. Bevilacqua, A. Regueira, M. Mauricio, J.M. Lema, M. Carballa, Spain</p>	<p><b>BioH<sub>2</sub> and bioCH<sub>4</sub> recovery by recirculated temperature-phased anaerobic co-digestion of food waste and paper waste</b></p> <p>Y. Qin, L. Li, J. Wu, B. Xiao, T. Hojo, K. Kubota, Y. Li, Japan</p>
14.30-14.45	<p><b>Using kinetic isotope effect to evaluate the significance of the sequential and parallel steps: formation of microbial consortium during reversible anaerobic methane oxidation coupled with sulfate reduction</b></p> <p>V.A. Vavilin, L.Y. Lokshina, S.V. Rytov, Russian Federation</p>	<p><b>Caproic acid production: process development, product recovery, microbiome characterization and techno-economic analysis</b></p> <p>P. Oleskowicz-Popiel, M. Lezyk, A. Duber, F. Brodowski, E. Jankowska, F. Walkiewicz, N. Gutowska, R. Zagrodnik, Poland</p>	<p><b>Enhanced anaerobic digestion of waste activated sludge using sludge incineration bottom ash</b></p> <p>Y. Shen, C. Yin, N. Zhu, China</p>

# Evaluation of protein composition influence on yields and selectivity of volatile fatty acids production

**Bevilacqua, R., A. Regueira, M. Mauricio, J.M. Lema, M. Carballa**

**Department of Chemical Engineering, Institute of Technology, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain - [riccardo.bevilacqua@usc.es](mailto:riccardo.bevilacqua@usc.es)**

**Abstract:** The influence of protein composition on volatile fatty acids (VFA) production is not clear and need to be further studied to valorise protein residual streams. For this purpose, two continuous stirred tank reactors were inoculated with acidogenic biomass and fed with two proteins (casein and gelatin) having different amino acids compositions. Casein showed a higher acidification degree and double the VFA productivity of gelatin, despite its imbalance between electron acceptor amino acids and donors. Experimental VFA spectra highlighted n-butyric acid as a major product for casein and propionic acid for gelatin. Finally, we hypothesised preferential consumption for some amino acids (AA), with the experimental results confirming it. For this reason, previous models based on fixed Stickland stoichiometry could be inaccurate at describing proteins degradation. This knowledge helps to better predict the outcome of the process when fermenting protein rich substrates.

**Keywords:** mixed culture fermentation; substrate influence; VFA production

**INTRODUCTION:** An appealing alternative to biomethane generation is found in the intermediate products of the fermentation: organic acids, ethanol and hydrogen. Their value lies in their countless applications as precursors for more complex compounds (Bathia et al., 2017). VFA can be potentially obtained from renewable sources, through anaerobic fermentation of organic wastes and sidestreams. The process has been studied for what concerns the degradation of sugars (especially glucose). Little is known about proteins fermentation: Ramsay and Pullammanappallil (2001) compiled a stoichiometric matrix of amino acids degradation based on Stickland reactions, proposing it was sufficient to describe a generic protein fermentation process. Even though it can be used to preliminarily evaluate VFA selectivity, it is contradicted by the experimental results of Breure and Van Andel (1984), through which it is possible to appreciate the influence of pH. Having a better knowledge on the process should allow for predicting and driving the process towards target VFA composition of the effluent. For this purpose, this study is focused on evaluating protein composition influence on the fermentation outcome, VFA selectivity in particular.

**MATERIALS AND METHODS:** Two continuous stirred tank reactors of 1 L working volume were used. Both CSTRs were maintained at 25°C. Hydraulic retention time was adjusted to 1.0 - 1.5 days. Proteins were fed to the CSTRs in the same concentration of 8.0 g COD/L as sole carbon source of the influent, leading to different OLR: 8.0 g COD/L·d for casein and 5.3 g COD/L·d for gelatin. Their composition in AA is described in table 1. No pH control systems were implemented: neutral values between 7.0 and 7.5 were obtained by sparging nitrogen ( $\approx 10$  mL/min), also meant to remove the hydrogen from the liquid phase. The reactors were inoculated with acidogenic biomass coming from a lab scale fermenter (VSS content of 1.0 g/L). Average biomass concentration obtained oscillated between 0.3 and 0.4 g/L in both CSTRs.

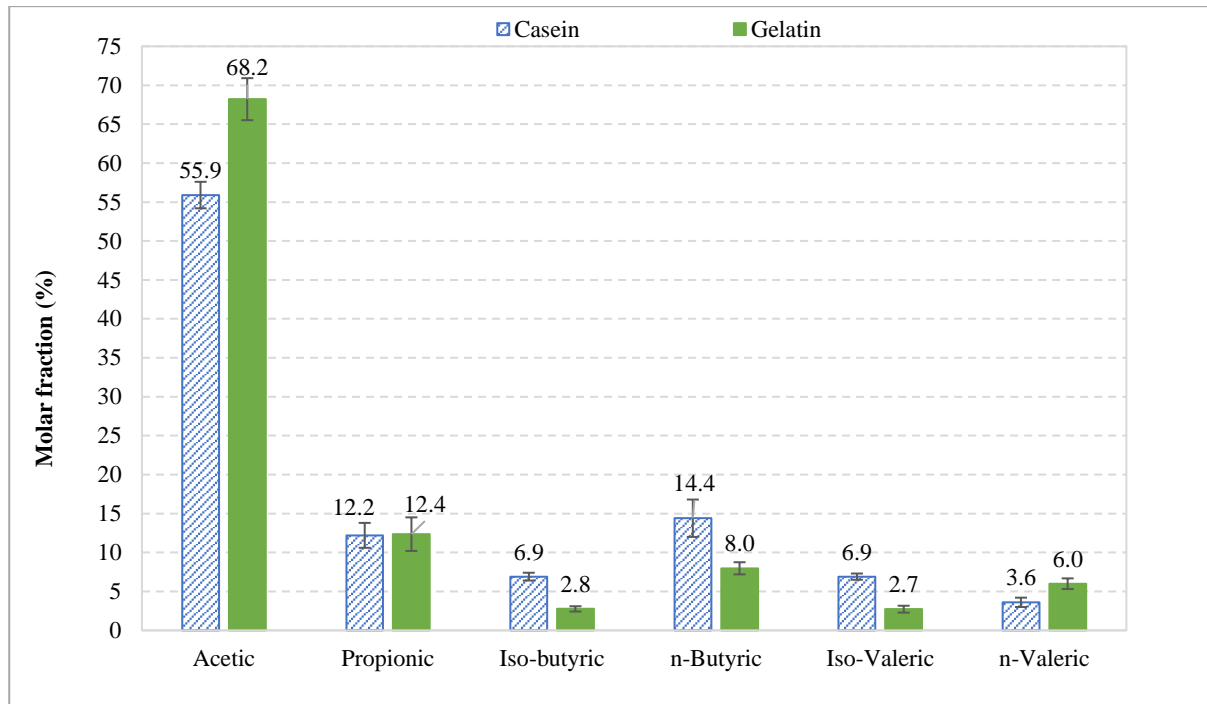
Standard analytical methods were used to monitor the conditions of the reactors. VFAs from C2 to C7 were measured through gas chromatography as with the gas composition of the headspace volumes.

**Table 1.** nominal AA abundancies of the chosen proteins with related VFA resulting of their degradation

Nominal molar composition (% mol)			
AA	Casein (Brunner, 1977)	Gelatin (Eastoe, 1955)	Stickland-related VFA(s)
Aspartic acid	6.52	5.02	Acetic
Glutamic acid	19.4	7.99	Acetic/n-butyric
Serine	7.73	3.93	Acetic
Glycine	3.05	37.3	Acetic
Histidine	2.63	0.55	Acetic/n-butyric
Arginine	2.79	5.06	Ac/prop/n-val
Threonine	3.95	1.87	Acetic/n-butyric
Alanine	4.00	12.5	Acetic
Proline	11.4	14.5	Ac/prop/n-val
Tyrosine	4.31	0.15	Acetic/aromatic
Valine	6.73	2.22	Iso-butyric
Isoleucine	5.58	1.33	Iso-valeric
Leucine	8.78	2.63	Iso-valeric
Phenylalanine	4.10	1.40	Aromatic
Lysine	6.42	2.95	Acetic/n-butyric
Methionine	2.58	0.58	Propionic

**RESULTS AND CONCLUSIONS:** the two reactors were continuously operated and fed with casein and gelatin for more than 100 days. Casein reactor reached a close-to-steady state in 30 days of operation. Afterwards, no methanisation was observed. Average acidification degree was close to 50% of the influent total COD. Gelatin reactor reached stability in 15 days. As with the casein reactor, no methanisation was detected. To increase the acidification degree from the initial 10% to 40% (in COD basis henceforth) several strategies were implemented: increasing HRT, cross inoculating with anaerobic reactor biomass and adding selenium to the feedstock. Still, casein reactor performed better at transforming the substrate to VFA: it reached a productivity of  $4.06 \pm 0.51$  g O<sub>2</sub>/L·d, while gelatin one reached  $2.12 \pm 0.21$  g O<sub>2</sub>/L·d.

Furthermore, VFA composition of the effluents produced during the experiments is different for the two reactors (Figure 1).



**Figure 1.** VFA spectra of the chosen proteins, shown as molar fraction of each detected organic acid

Fermentation of both proteins leads to VFA spectra dominated by acetic acid, especially gelatin (70% of total of VFA). Relevant differences are found in the other products: n-butyric acid is the second most abundant casein VFA, while in the case of gelatin fermentation it is propionic. Iso-acids have higher relevance in casein spectrum while the opposite applies to n-valeric acid.

Considering the experimental results, it was determined that proteins with different composition in AA lead to different acidification degrees. We hypothesised that either fixed Stickland stoichiometry is not suitable for describing AA degradation or redox roles of some AA were assumed incorrectly. In fact, casein is more easily acidified than gelatin despite its imbalance between electron acceptors and donors (Table 2). Moreover, a preferential consumption of a selected few AA over the others could have affected the process: gelatin fermentation might be hindered by its high content of glycine (Table 1), whose uptake is inhibited for some bacterial species until all proline is consumed (Barker, 1981). Furthermore, glycine degradation depends on a specific reductase enzyme, which requires selenium to be produced. Despite selenium dioxide (1  $\mu$ M) addition to the feedstock, no increase of the acidification degree was observed, suggesting that glycine was not being consistently converted.

**Table 2.** Amino acids distribution in redox roles based on Stickland reactions

AA redox roles - molar basis					
De Vladar - 2012			Ramsay - 1997		
Role	Casein	Gelatin	Role	Casein	Gelatin
Acceptor	21.0	56.8	Acceptor	14.5	51.8
A/D	20.0	9.25	A/D	21.1	6.06
Donor	59.0	33.9	Donor	62.1	40.9
			Uncoupled	2.25	1.29

Influence of AA abundancies was also identified in terms of selectivity, hinting to preferential consumption of some over the others:

- Casein higher abundancies of glutamate, lysine, histidine and threonine lead to higher values of butyric acid than for gelatin (Table 1). COD values for both proteins reflects the stoichiometric prediction when considering the experimental acidification degrees.
- Valine consumption is higher than the average AA consumption for both proteins, based on the experimental concentrations obtained for iso-butyric acid. When comparing the proteins composition (table 1), it appears that preferential consumption of valine is stronger for gelatin.
- Iso-valeric acid production is lower than expected, possibly indicating that leucine and isoleucine degradation is subordinated to the abundancy of other AAs.
- Propionic and n-valeric acids depends on arginine, proline and methionine consumption, but their proportions do not reflect stoichiometric values: the first shows higher concentration than the second (COD basis). This suggests the need for further validation of their conversion reactions.

In general, a preferential consumption of some AAs can explain the deviations from theoretical VFA spectra predicted through the fixed Stickland stoichiometry, especially when considering that it assumes 100% of AA consumption, against the 40-50% of acidification degree obtained experimentally.

In conclusion, this study successfully evaluated the influence the amino acids abundancies on mixed culture fermentation processes, both in terms of acidification degree and selectivity. Possible discrepancies in AA roles with previous literature and mechanisms of preferential consumption were highlighted. Using this knowledge, prediction of the VFA spectra should be feasible and could help drive the process towards desired products through optimal blending of different protein-rich sidestreams. Still, mechanisms related to preferential consumption have to be further investigated to improve the prediction. Study of pH influence on the process is the next step, with the objective of optimising the process adjusting the operational conditions.

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