Science of enzyme dosing in anaerobic digestion

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Introduction

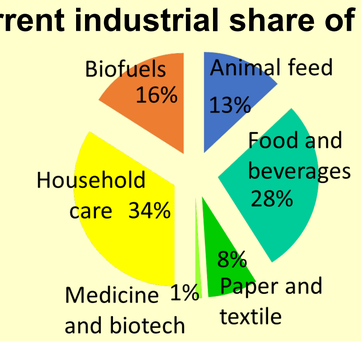
Why enzymes?

Biological catalysts
- Reduced activation energy of reactions
- Specificity
- Environment friendly

Energy saving
- Stable and work in mild conditions

Cost saving
- Less chemicals
- CAPEX saving for direct additions
- Cheap industrial enzymes

Current industrial share of enzymes



What about waste and wastewater?

…and in anaerobic digestion?

Enzymes in anaerobic digestion

Enzymes have been successfully proven to enhance performance of anaerobic digestion (AD) treating a variety of feedstocks; e.g. energy crops, municipal organic waste, sewage sludge.Several studies have shown that enzymatic pre-treatment can enhance the hydrolysis step of the AD process[1, 2, 3], although only some of them suggested real improvements in biogas yield.[4, 5, 6]

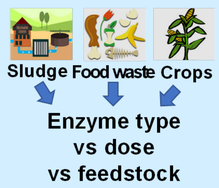
Increase in biogas yields were also reported for enzyme direct addition[4, 5, 7] coupled with additional benefits, such as increased dewaterability and solid removal.

Aim, objectives & methodology

Aim of this work was to assess the feasibility of using enzymes in anaerobic digestion, with the following objectives:

1. Enzyme tailoring

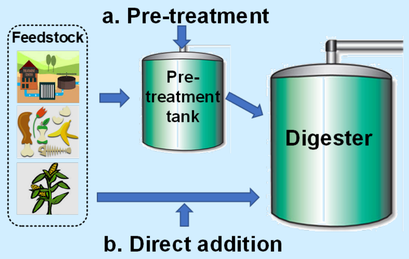
Determine an easy method to select the best enzyme vs feedstock vs dose combination



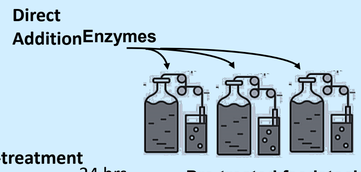
Solubility test
Substrates (250 g, 150 g and 100 g for sewage sludge, energy crops and food waste, respectively) were mixed with the required amounts of enzymes as a liquid solution and incubated at 37˚C in 500 mL flasks. Control samples without enzymes were maintained at 37°C for an equal amount of time. At the end of the 24 hours the increase of soluble COD was used to identify the most suitable enzyme.

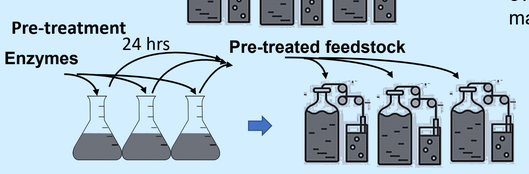
2. Enzyme application

Identify the different effects and implications of the two treatments



AD batch test
Enzymes were used in batch test with the three feedstock, following a 24 hours pre-treatment or as a direct addition to the reactor and biogas production was compared to those of control or control maintained at 37°C for 24 hours.
Direct addition were also done using pre- and post- digestion composition.

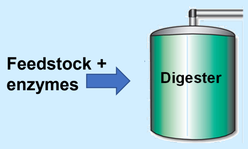




3. Long-term use implications

Evaluate the long-term impact of direct additions

Direct addition:
a.Strategy
b. Long-term impact



Semi-continuous digestion
1 l reactors with a 700 ml working volume, using AMPTS II automated system (Bioprocess control, Lund, Sweden) and sludge. The reactors were maintained at 38°C and fed every two days at an OLR of 2.69 gVS/l·day, allowing and a 16-day hydraulic residence time (HRT). The methane content was measured using a SERVOPRO1400 CH4 gas analyser (Servomex, UK). Enzymes were added to the systems once every HRT, mixed to the feeding material (4200U/gTS).

Table 1. Enzyme doses used for solubility tests and batch tests

Enzyme	Activity (U/g)	Supplier dose	Waste feestock	Solubility test (U/gTS)	Batch test Pre-treat. (U/gTS)	Batch test Direct addition (U/gTS) Pre[3]	Post[3]
Amylase MT-3K[2]	3000	0.07-0.2 ml/100ml	Sludge	820			
			En. crops	50			570
			FW	2800	2800	60	940
Cellulase ACx8000L[2]	8000	0.02-0.03ml/100ml	Sludge	900			
			En. crops	100			
			FW	200			
Lipase NL-GX[2]	100	0.1 % w/w	Sludge	40			
			En. crops	5			
			FW	0.5	0.5	0.5	
Protease BS-L[2]	900	0.1 % of protein	Sludge	130			5
			En. crops	0.5			0.5
			FW	4		4	10
Lipase LIP200[1]	2E-05	30-100g per ton of feedstuff	Sludge	1170	1170	1170	5
			En. crops	305			
			FW	2800			
Cellulase CEL200[1]	2E-06	50-300g per ton of material	Sludge	2100	2100	2100	3290
			En. crops	7920		7920	5340
			FW	8000			8000

Enzyme selection
The enzymes and the doses used in the batch systems were selected following the materials characterisation (pre- and post-digestion) and manufacturer recommendation (Summary reported in table 1). All concentrations are reported as U/gTS.

[1]Sinobios (China)
[2]Enzyme Supplies (Oxford, UK)
[3] Pre and post refer to doses of enzymes calculated from the material composition before anaerobic digestion (ingestate) or after anaerobic digestion (digestate).

Results

1, 2. Enzyme tailoring and applications

Although useful as a guidance, the solubility tests does not always compares with the results obtained from batch tests.
Direct addition and pre-treatment showed different optimisation results, the former in biogas quality, the latter in biogas yields.
The action mechanisms for the pre-treatment is an enhancement of the hydrolysis step due to direct solubilisation of the organic matter is expected.
Whereas direct addition is likely to impact also on the composition of microbial community.
The two processes work in a different way and hence, tailoring needs to be done in different ways.

Table 2. Results from solubility tests and batch tests

	Dose U/gTS	Soluble COD increase after 24 hours g/gVS	Pre-treatment Cumulative biogas ml/gVS added	Methane content %	Direct addition Cumulative biogas ml/gVS added	Methane content %
Sludge						
Amylase MT-3K	820	12 ± 1.5				
Cellulase ACx8000L	900	81 ± 8.3				
Lipase NL-GX	40	4 ± 2.7				
Protease BS-L	130	46 ± 6.3				
Lipase LIP 200	1170	7 ± 4.2	148 * ± 5.0	71%	263 ± 4.0	45%
Cellulase CEL 200	2100	159 ± 16	131* ± 9.5	70%	250 ± 8.5	52%
CEL 200 + LIP 200	--	--	135* ± 18	81%	255 ± 4.0	63%
Control	--	--			246 ± 6.9	47%
Control*	--	--	124* ± 19	76%		
Energy crops						
Amylase MT-3K	50	0				
Cellulase ACx8000L	100	39 ± 0.4				
Lipase NL-GX	5	0				
Protease BS-L	0.5	0				
Lipase LIP 200	310	0				
Cellulase CEL 200	9920	41 ± 2.1			687 ± 8.2	59%
Control	--	--			509 ± 50	54%
Food Waste						
Amylase MT-3K	2800	660 ± 140	295** ± 54	52%	288[2] ± 9.3	66%
Cellulase ACx8000L	200	14 ± 12.4				
Lipase NL-GX	0.5	391 ± 221			181 ± 11	60%
Protease BS-L	4	4 ± 0.1			184 ± 0.6	62%
Lipase LIP 200	2800	235 ± 111	452** ± 17	56%		
Cellulase CEL 200	8000	281 ± 19.7				
Amylase + Lipase	--	--	109** ± 43	59%	255 ± 1.8	68%
Control	--	--			167 ± 3.5	59%
Control**	--	--	475** ± 7.4	60%		

Table 3. Batch test results of direct addition, following pre- and post digestion tailoring

		Pre-digestion tailoring		Post-digestion tailoring	
	Doses U/gTS Pre-Post	Biogas increase % (p-value)	Methane content % (p-value)	Biogas increase % (p-value)	Methane content % (p-value)
Sludge					
Cellulase	2100-3290	1.2 (0.95)	52 (0.44)	12 (0.42)	66 (0.52)
Lipase	1170-5	6.3 (0.46)	45 (0.78)	0.5 (0.46)	65 (0.41)
Protease	0-5	-	-	8.6 (0.42)	66 (0.49)
Energy crops					
Amylase	0-570	-	-	23 (5.7E-06)	56 (8.5E-06)
Cellulase	9920-5340	26 (8.0E-06)	40 (4.4E-07)	10 (0.054)	57 (2.3E-06)
Protease	0-0.5	-	-	7.8 (0.225)	56 (7.9E-06)
Food Waste					
Amylase	60-940	42 (1.1E-06)	66 (0.01)	21 (0.02)	75 (1.8E-06)
Cellulase	0-8000	-	-	9.5 (0.02)	70 (7.1E-05)
Lipase	0.5-0	7.6 (0.14)	60 (0.52)	-	-
Protease	4-10	9.1 (0.08)	63 (0.24)	14 (0.03)	75 (2.1E-05)

It is clear from the data reported in table 2 that an increase in enzyme/substrate ratio will lead to an increase in reaction rate as more enzyme will be available to interact with the substrate. However, the system will reach a saturation point after which, a higher amount of enzyme will necessarily produce and increase in reaction yields, as the enzyme concentration will not be any more the limiting factor in the reaction. Therefore, an enzyme optimal concentration value exists for each reaction. This optimal value can be identified using solubilisation test and batch digestion. A comprehensive testing of concentrations should be done for each enzyme to assess this saturation point to identify the optimal combination of concentration vs solubilisation and avoid overdosing and ineffective use of the enzymes dosed..

3. Long-term use implications

Data from the batch tests with sludge were validated during the semi-continuous experiments were pre- and post-digestion doses of Cellulase CELL 200 (2100U/gTS and 4200U/gTS respectively) were added to the reactors (Figure 1). The results showed positive long-term effects on AD process. Both enzyme doses (pre- and post-digestion) produced significantly larger quantity of biogas (p-value of 2.06E-21 for pre-digestion, 9.94E-32 for post-digestion). Higher gas productions and methane yields were obtained in enzyme-added anaerobic reactors also by Recktenwald et al. (2008)[7]. The same authors showed increased dewaterability in enzyme dosed reactors, due to enhanced degradation of extracellular polymeric substances, in particular of the carbonaceous matter. Ayol et al. (2008)[4] reported that this enhanced degradation of the EPS matrix will improve sludge solids solubilisation and hence the formation of enzyme-substrate complexes. In addition to organic solubilisation and increased hydrolytic activity, direct additions have also proved to increase process stability and methanogenic activity.

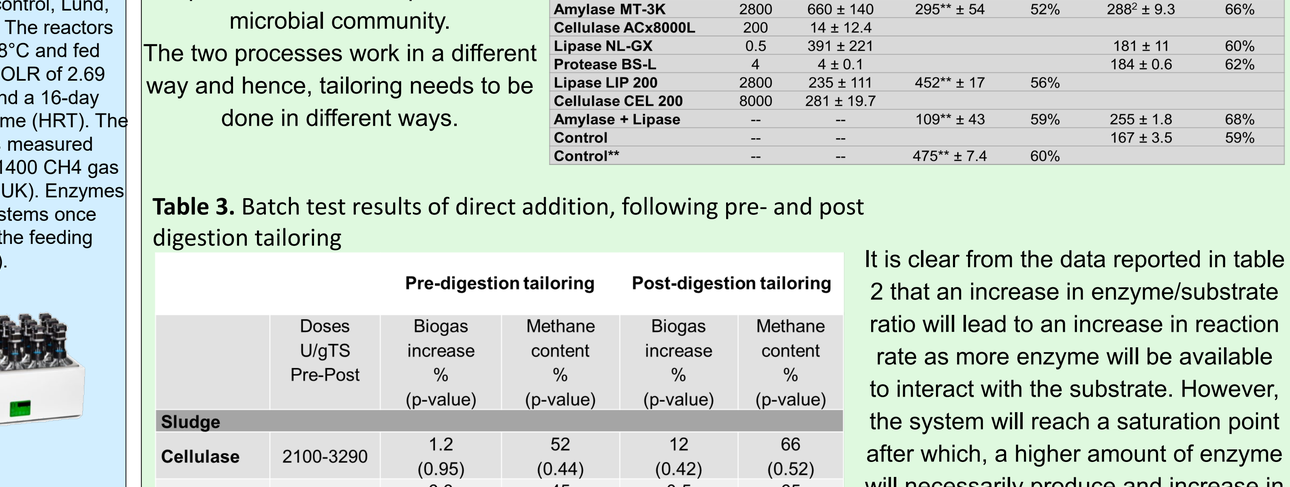


Figure 1. : Long-term effect of enzyme addition using Pre-digestion and Post-digestion tailored dose of cellulase (A) biogas content (Nm/gVS) and (B) methane percentage (%).

Conclusions

Enzymatic treatment in the AD sector is being underused mainly due to the cost of the enzymes itself and a poor understanding of the enzyme action and their long-term impact on the process. Both enzymatic treatment methods enhanced biogas production, although significant difference in performance of direct addition of enzymes and enzymatic pre-treatment was observed. Our research proved that AD performance improvement by enzyme dosing can be economically feasible by using industrial enzymes and tailoring the enzyme type an dose to the nature of the feedstock treated. The long-term impact is beneficial mainly through the improvement in biogas quality.

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