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Influence of temperature and pH on enzyme activity in the biogas process

Enzyme supplementation is often used in agricultural biogas plants to accelerate degradation of crop fibre (e.g. cellulose and hemi-cellulose) and thus increase biogas yield. However, the efficacy of such enzyme supplementation has been insufficiently tested under laboratory conditions. In order to systematically investigate factors influencing enzyme activity, enzymatic hydrolysis experiments were conducted on maize straw at the University of Hohenheim to test the efficacy of commercial enzyme supplementation under controlled conditions outwith the biogas production process.

Key words
Biogas, enzymes, energy crops, hydrolysis

Abstract

Enzyme additions are often used in biogas plants, to increase the degradation of polysaccharides and the biogas yield. So far, the effect of these commercial enzymes was only insufficient tested. The University of Hohenheim conducted enzymatic hydrolyses on Maize straw to test their activity and the factors which influence it.

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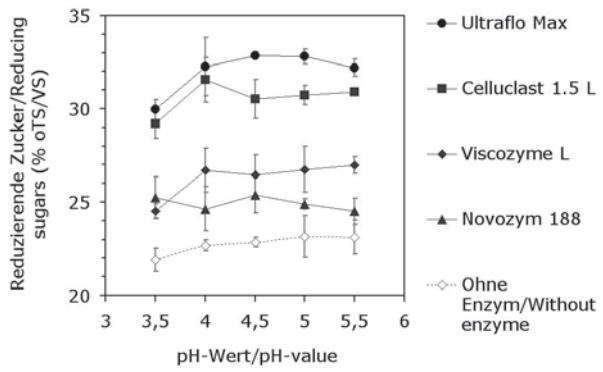
In Germany a large number of agricultural biogas plants are run through co-fermentation of energy crops and liquid manure. The fibres within the energy crop plants are difficult to degrade, or can only be degraded slowly, by anaerobic bacteria. Improved digestion of these substrate fractions could increase the degree of degradation and therefore methane yield. For this reason fibre-decomposing enzyme preparations are often recommended for use in the biogas process. These enzymes are aimed at accelerating degradation of the plant fibre components and thus increasing biogas yield by degrading polysaccharides to soluble sugars. The actual efficacy of these added enzymes on the crop substrate has been tested and produced inconsistent results in fermentation experiments by various research groups [1, 2, 3]. Many biochemical actions take place within the biogas process and this makes difficult the testing of enzyme efficacy. The experiments presented here investigated the effica-

Table 1

Enzymname Enzyme name	**Hauptaktivität** Main activity	**Mikroorganismen** Microorganisms	**Anwendung** Application	**Temperatur-optimum (°C)** Temperature optimum (°C)	**pH-Optimum bzw. pH-Bereich** pH optimum / pH area
Celluclast 1.5L	Zellulase	*Trichoderma reesei*	Lebensmittel	65	5
Novozym 188	Zellobiase	*Aspergillus niger*	Lebensmittel	55	5,5
Novozym 342	Zellulase	*Humicola sp.*	Textil	40 - 65	7,5
UltrafloMax	β – Glucanase, Xylanase	*keine Angabe*	Bierfiltrierung	55°C	4,5 - 6,5
Viscozyme L	Arabinase, Zellulase, Hemizellulase, Xylanase	*Aspergillus aculeatus*	Lebensmittel	25 - 55	3,3 – 5,5

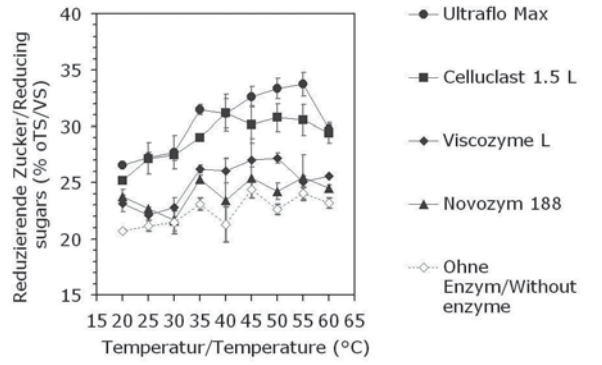
Activities and properties of the tested enzymes (Source: Novozymes – Product Sheet)

Fig. 1



Reducing sugar release after 24 hours of maize straw hydrolysis at different pH values between 3,5 and 5,5; trial in water bath at 50 ºC

Fig. 2



Effect of temperature on reducing sugars content after 24 hours hydrolysis of maize straw at pH 4,5

cy of commercial enzyme preparations in degrading maize straw cellulose and hemi-cellulose. Two series of experiments thereby investigated the influence of temperature and pH on enzymatic hydrolysis. As indicator of enzyme efficacy, release of soluble sugars during enzymatic hydrolysis in water medium was recorded via photometric analysis.

Material and methods

Maize material (variety: Gavott) was used as substrate for the experiments and this was separated into two fractions (cob and straw) after harvest with grain at milky stage. The cob fraction showed a high content of starches and soluble sugars. Contrary to this was the relatively high proportion of difficult to degrade fractions (above all, cellulose and hemi-cellulose) in the remainder of the maize plant (the maize straw). The experiments presented here were conducted with this fibrous remainder.

The maize plant remainder was ground (fibre length < 3 mm) and frozen until required for tests. Enzymes added were provided by Novozymes A/S, Bagsvaerd, Denmark. The enzymes, their characteristics and manufacturer‘s information are presented in table 1. Amount of enzyme preparation to be added was put at 3% enzyme solution in all experiments and based on the organic dry matter (ODM) of the substrate under test.

The enzymatic hydrolysis was carried out in a vibration water bath at vibration speed of 60 rpm with 1.8 g fresh ground maize straw and 10 mL citrate buffer 0.1 M (pH range 3.5 to 6) or phosphate buffer 0.1 M (pH 7) added in glass flasks. The enzymatic hydrolysis lasted 24 hours. Temperature was varied between 20 and 60°C and pH between 3.5 and 8.

During the enzymatic hydrolysis, enzyme action degraded cellulose and hemi-cellulose sugar chains in the maize straw to soluble sugars with reducing characteristic (reducing sugars) that were marked with the colour reagent 3.5 dinitrosalicylic acid (DNS) according to the method developed by MILLER in 1959 with the simplified formulation of the reagent solution from WOOD et al. 1988. For this, samples were filtered and diluted 1:50 in volumetric flasks after enzymatic hydrolysis. 2 mL from the filtrated sample was added to 3 mL DNS preparation in a test tube, heated for exactly 15 minutes on a hotplate at approx. 95 °C and cooled immediately afterwards. Following 30 minutes waiting to allow colour stabilisation, absorbance measurement of the samples was conducted at a wavelength of 640nm in the photometer (SHIMADZU UV Mini 1240 UV-VIS-Spectrophotometer).

A glucose calibration curve, with increasing glucose concentrations between 0 and 1 g/L at 0.1 g/L intervals, was depicted to determine the amount of reducing sugars. The extinction of the glucose solution varied in the range 0.0 to 0.5 and measurement results in the range 0.1 to 0.3. In a preliminary test the enzyme content of reducing sugars was analysed. This was found to be lower than 0.5% based on the ODM and could therefore be ignored in the results.

Results of the laboratory experiments

In a first series of experiments pH of samples before addition of enzymes was set at between 3.5 and 5.5 by citrate buffer. According to the manufacturer’s information the activity optimum for most of the enzymes investigated lay in this range. The test temperature was 50°C with results presented in fig. 1.

With a further increase in pH in the enzymatic hydrolysis a massive acid accumulation occurred as from pH 6. This reduced pH by up to three units over 24 hours. The accumulation of up to 1000 ppm acetic acid determined by analysis of the hydrolysate at pH 7 indicated increased bacterial activity at the higher pH (6 and 7) whereby the produced sugar was metabolised to carbonic acids. In that the laboratory method to suppress microbial activity (preliminary heating of substrate and medium, Tyndallisation) showed no success, a limited microbial contamination of the enzymes must be assumed in that only those were left out of the treatment. For the experiments with the enzyme Novozym 342 at a higher pH of 7, the additi-

on of toxic sodium azide (in a concentration of 1% of substrate ODM) was tested to suppress bacterial development. Even in low concentrations the sodium azide had the effect of blocking the respiratory chain and thus causing the microorganisms to die off [4]. As a result of the addition in the tests pH was stabilised and content of reducing sugars rose from 20.85 to 28.05% of substrate ODM.

In a second series of experiments the pH was set at 4.5 and the temperature varied between 20 and 60°C which covers the complete temperature range of biogas plants currently. The amount of reducing sugar produced is presented in fig. 2.

Discussion

The content of soluble sugars in the tested "remaining maize material" substrate before enzyme application was approx. 22 to 23% of ODM, representing values given in the literature [5, 6]. The total amount of soluble sugars and polysaccharides in the substrate was more than 80% of the ODM. Through enzymatic hydrolysis it was possible to increase concentrations of soluble sugar compounds in all tested variants. With a pH of 4.5 and a process temperature of 55°C, however, only a maximum of 33% reducing sugars in ODS could be captured, even although the carbohydrate fraction of the initial value led to expectations of much higher concentrations. Compared with the untreated variant the relative proportion of reducing sugar is only increased by up to 40% although the applied concentration of enzyme was approx. 100 times higher than in practical conditions [2]. The already high sugar concentration in the medium at the beginning of enzymatic hydrolysis could have had an inhibiting effect on enzyme efficacy [7]. To avoid this situation in further experiments, soluble sugars in the initial substrate should be rinsed out beforehand [8].

Optimum pH for the commonly applied fungal origin enzymes lies between 4.0 and 6.0 [9] whereby biogas fermentation takes place between 7.0 and 8.5 pH in practice. This therefore raises the question as to whether fungal enzymes retain their efficacy at higher pH levels. There should thus be further tests wherein activity of sugar degrading microorganisms is prevented by addition of sodium azide thus allowing higher pH levels.

The tested enzymes were also effective at the usual biogas process temperatures of from 30 to 40°C. However, a better effect was achieved at a higher temperature of 50°C which agreed with the manufacturer's recommendations. Enzyme activities were reduced at 60°C. It is known that enzymes show a higher efficiency at higher temperatures although inactivated by temperatures that are too high [9].

For increasing enzymatic hydrolysis efficiency, the crop substrate should be perhaps processed through a preliminary physicochemical treatment (e.g. preheating and addition of acid or alkali) in order to break down fibre structure [8]. Positive or negative interactions taking place between added enzymes and living microorganisms that influence enzyme efficacy in the biogas process can play a great role and therefore should be investigated in the future.

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