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Deactivating Fusarium Spores throughout Anaerobic Fermentation in Biogas Plants

A Prospect

Fusarium (the most harmful grain fungus in the field, known as fusarium head blight) and its poisonous product, catabolic mycotoxin DON (Deoxynivalenol) are known for their damaging effects. Due to this, the most feasible, environmentally compatible and economical disposal option are being researched in a cooperative project, where deactivating the fungus and reducing its mycotoxin are in the foreground.

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Keywords

Anaerobic fermentation, biogas, Deoxynivalenol (DON), energy production, Fusarium, deactivation, mycotoxin reduction

Annually there are approximately 3.8 Mio t of grain contaminated by fungi. Under preferential ambient conditions (15 to 20°C), Fusarium produces intermediate catabolic products. The FAO estimates 25% of the world's grain crop is contaminated with intermediate catabolic products [1]. Deoxynivalenol (DON) is the most familiar toxin and known to provoke harmful anorexia and emesis, caused by short- or long-term intake.

Legal situation within the EU

To prevent these toxins from being spread into the human food chain, the European Community launched a regulation, providing maximum levels of contaminants [2]. The amendment is basically focusing inter alia on Deoxynivalenol (DON), Zearalenone (ZEA) and Fumonisins. For DON, the following maximum load for unprocessed cereal is authorized with 1250 µg/kg. Due to these official requirements a safe disposal of the contaminated wheat charges is demanded. The main task of a current research approach at Hohenheim University is to investigate the prerequisites of a potential deactivation of Fusarium spores in a fermenting process. Ruminal micro organisms are capable to inactivate defined amounts of Deoxynivalenol (DON) by transformation it into its 24 times less cell-toxical Deepoxynivalenol (DOM-1). Analysis proved an entirely transformation of 10 mg DON into DOM-1 throughout the so-called ruminant detoxification mechanism within 24 hours [3].

Objective

Several test series have been conducted according to VDI directive 4630 [4]. The determination of microbiological activity rate was proven at a bench scale unit for charges of contaminated wheat of whole grain and as ground flour in discontinuous batch processes. The investigations within this cooperative project are as follows:

- Potential of biogas generation: to detect microbiological inhibition of the fermentation process by contaminated substrates
- Germination capacity of the Fusarium spores: to abate the infectious field load by the applying fermentation residues on new crops
- Monitoring of toxins: to break the path of infection, re-infection can not be excluded ("carry over-effect")

Fusarium contaminated wheat batches

To investigate the potential deactivation of Fusarium and an aborticide of Fusarium during the fermentation process, a sample of inoculated wheat was used as test material. After harvesting the material was separated by a cyclone to realize homogenous basic material without contraries (rudimentary developed kernels and glumes). The derived material was used in the trial as ground material and as entire kernels.

Midget batch trial - Hohenheim Biogas Yield Test (HBT)

One option for the evaluation of the biogas potential is to determine the appearing CH_4 yield of standardly inoculated manure, while the specimen is conducting the HBT (VDI 4630; DIN 38414 part 8) [5]. Arranging the established approach by the use of midget biogas fermenter-vessels (glass samplers with ml-scale), 30 ml charge of standard manure and 500 mg test substrate are commonly used. The qualitative gas analysis via infrared sensor delivers a quantitative determination of the composed gas a-

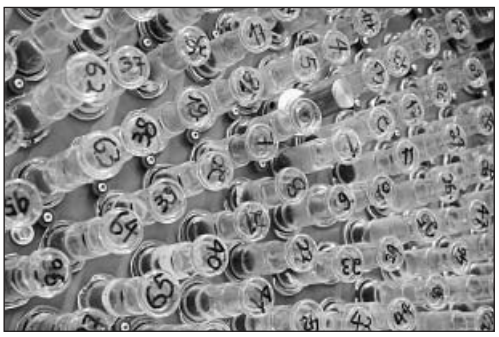

Fig. 1: Arrangement of flask samplers in an incubator

mount in combination with the intrinsic substrate potential. Consequently every inhibition of microbiological degradation throughout the whole fermentation process (35 days) can be revealed [6] (*Fig. 1*).

Fusarium detection by identification

A microbiological reconditioning and detection of Fusarium is tested with Schmidt agar plates after fermentation by a visual control of macro or micro conidia [7]. The characteristics of the measurand are documented for the entire runtime of the trial; schedule of flask samplers dismounting at retention periods of 0.5 to 36 days. The quantification of affection by moulds is determined before and after fermentation trials with the revealing of concurrent infection rate (IR) on culture media. Nine samples are taken from the incubator at aforementioned times. After plating, samples are stored at 28°C and a minimum residence time of eight days in a micro-aerophil incubator. Microscopic analysis to detect Fusarium and its spores are accomplished from the first up to the 12th week post experimentum. Visual criteria for a positive classification are reddish to yellowish mycelia and crescent-shaped septed spores.

Interpretation of the biogas process

The separate interpretation of appearing fermentation run and the specific methane yield leads to the conclusion that the kinetic as an indicator of the fermentation quality reveals no inhibition. With the start of the fermentation trial the population of common anaerobic bacteria is marginal and solitary fractionally adapted. Until the fourth day the development phase is found secluded. There the micro organisms reach their highest performance, until their activity slows down due to the exhausted nutrition status in the ambient media. Using the specific methane yield as an indicator to reveal potential interactions of contaminated substrates, yield cuts are consequently used for identification. According to other curve developments, the

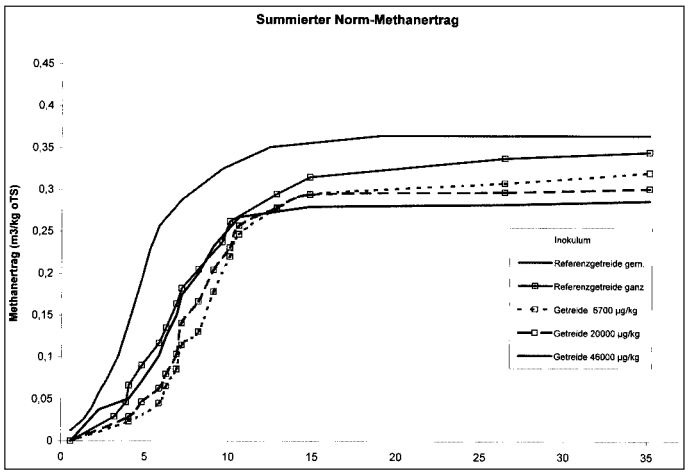

Fig. 2: Mesophilic anaerobic fermentation, inoculation of cereal by Fusarium

set of "certified reference material, ground" shows a relatively higher yield (*Fig. 2*). This effect can partly be explained by conditions of an eased enzymatic activity when grounded material is decomposing. Another explanation can be found in the decline of germane ingredients: Thousand-seed weight differs from mean by 43g (regular kernel), from contaminated kernels by 30g from mean [8].

The Weender/van Soest analysis showed reduced contents of starch and sugar for contaminated substrates. However, the biogas forming process is – based on the biogas forming potential - not affected adversely by adding contaminated material. The addition of microbiologically contaminated charge showed max. loads of 46.000 µg/kg.

Microbiological interpretation

An aborticide of Fusarium by means of the biogas fermentation in the two temperature sets can be underlined from first results, see *Table 1*. The infection of unfermented material comprises a rate of 100 percent, caused by an expeditious domination of Fusarium on inoculated kernels. This was found evident after a retention time of 0.5 days by means of aforementioned extraction and examination. The inactivation of Fusarium can be explained by the absence of oxygen. Other moulds were assigned to the group of total moulds (*Aspergillus flavus, Penecillium rouqueforti* and others).

Table 1: Temperature-time effect on infection rate (in %)

Mesophile fermentation: 37°C	**before fermentation**	**after 0,5 days of fermentation**	**after 12 days of fermentation**	**after 35 days of fermentation**
total moulds in %	100	50	40	10
thereof Fusarium in %	100	0	0	0
Thermophile fermentation: 53°C	**before fermentation**	**after 0,5 days of fermentation**	**after 12 days of fermentation**	**after 35 days of fermentation**
total moulds in %	100	0	0	0
yeast in %	0	100	100	100
thereof Fusarium in %	100	0	0	0

Outlook

First results of the interdisciplinary approach showed a realistic potential of Fusaria deactivation. The HBT batch trial demonstrated the biogas process steady under the conditions of contaminated charge supplement. These first results have to be verified by a further variation of basic process parameters (temperature, dry matter content of the process, fermentation process alternatives). A supplementary analysis of the metabolic procedure and its aforementioned parameters is imperative.

Literature

Books are marked with •

[1] FAO http://faostat.fao.org/faostat/collections?version=ext&hasbulk=0&subset=agriculture

[2] Official Journal of the European Union: Commission Regulation (EC) No. 856/2005 of amending Regulation (EC) No. 466/2001 as regards Fusarium Toxins; 06/2005

[3] *King, R., E. McQueen, D. Levesque and R. Greenhalgh*: Transformation of Deoxynivalenol (Vomitoxin) by Rumen Microorganisms. J. Agric. Food Chem. 32 (1984), pp. 1181-1 183

[4] VDI Richtlinie 4630: Fermentation of organic materials, characterization of the substrate, sampling, collection of material data, fermentation test. 2006

[5] DEV Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung, Gruppe S-Schlamm und Sedimente, Bestimmung des Faulverhaltens, DIN 38 414, Teil 8, 1985

[6] *Helfrich, D., und H. Oechsner*: Hohenheimer Biogasertragstest - Vergleich verschiedener Laborverfahren zur Vergärung von Biomasse. Landtechnik 58 (2003), H. 3, S. 148 - 149; ATF 9 (2003), H. 3, S. 27-30

[7] • *Petrini, L, und E. Orlando*: Schimmelpilze und deren Bestimmung. Bibliotheca Mycologica Band 194, Gebrüder Bornträger Verlag, Stuttgart, Berlin, 2002

[8] LfL, Bayern, http://www.lfl.bayern.de/labor_aktuell/artikel/08627/ (2006)