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# SURVIVAL OF PLANT PATHOGENS AND WEED SEEDS DURING ANAEROBIC DIGESTION

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## ABSTRACT

The survival of *Plasmodiophora brassicae* was studied at digestion temperatures of 35 °C and 55°C in laboratory batch digesters within one and two weeks, respectively. The study showed that digestion at 55 °C almost completely reduced the ability of infecting bait plants.
The seeds of *Rumex obtusifolius* and *Lycopersicon lycopersicum* (tomatoes) were completely destroyed at 55 °C and a digestion time of 14 days, in laboratory batch digesters.
The experiments carried out in a two stage pilot plant (Leach-Bed Solid Phase batch Digester for hydrolysis and a pulsating dynamic anaerobic filter for methanogenesis ) showed that hydrolytic conditions are mainly responsible for the killing of weed seeds. Some additional data on the qualities of different digested substrates are presented.

## KEYWORDS

Anaerobic digestion; plant pathogens; weed seeds; survival; compost quality; sanitation.

## INTRODUCTION

The Swiss waste act, which came into force in December 1990, demands separate collection of the organic fraction of municipal solid waste and an appropriate treatment . Therefore, the amount of biowaste with a low dry matter content and poor in structure has recently increased dramatically.

At the same time some composting plants working with open windrow systems caused odor problems due to the large amount of kitchen waste. Thus, anaerobic digestion is becoming more and more important, being the most suitable technology for treating the wet part, which is poor in structure. The latter is mainly responsible for odor problems in traditionally operated composting plants.

The breakthrough of anaerobic digestion depends largely on the marketability of its final products. In other words, the anaerobically obtained products have to fulfil the same quality standards as compost. Especially, they have to be free of viable weed seeds and phytopathogenic germs.

The theme of this study was to determine whether weed seeds and plant pathogens are able to survive in anaerobic digestion systems. This is of vital importance, because otherwise an aerobic post-treatment, with at least one thermophilic phase, would be necessary.

*Plasmodiophora brassicae*, causal agent of the clubroot disease of crucifers, is able to survive in aerobic composts (Bruns et al., 1990). Because of its heat resistance, *Plasmodiophora brassicae* was used as indicator organism (Vogtmann *et al.*, 1979) in order to prove the degree of hygienisation during composting. No information is available about the influence of anaerobic digestion on the viability of this fungus. Some results of first experiments are presented in this paper.

The effect of anaerobic digestion on the very resistant seeds of *Lycopersicon lycopersicum* (tomatoes) was studied. Furthermore, the survival of *Digitaria sanguinalis* and *Rumex obtusifolius* was studied in different digestion systems. This paper gives a short review on the obtained results.

## MATERIALS AND METHODS

### Plant Pathogens

Cabbage roots, infested with *P. brassicae*, were collected from a field after harvest and stored at 15 °C. Samples were then taken from the stock: one half was kept at 15 °C to control pathogenicity and the other half was treated in two litre laboratory batch digesters for one and two weeks, respectively. Two different digester charges were tested: the first was composed of 10% infested roots (placed in a nylon bag), 60% bio-waste and 30% digester inoculum; the second contained 70% infested roots and 30% digester inoculum. The digestion temperature was either 35 °C or 55 °C. After each digestion process, the roots were taken out of the bag, homogenized and mixed with autoclaved organic soil in a ratio of 1:10. Chinese cabbage was then used as bait plant. The percentage of plants with root galls was evaluated after 5 weeks.

Artificially added Weed Seeds

The survival of a series of weed seeds, such as *Digitaria sanguinalis* (millet), *Rumex obtusifolius and Lycopersicon lycopersicum* (tomatoes) was tested in laboratory scale batch digesters and in a two-stage digestion pilot plant with batch hydrolysis. Of each weed, 500 seeds were put into nylon tissue bags and placed either in the laboratory batch digesters or into the vessels of a two-stage digestion pilot plant. In the dynamic filter they were exposed to the liquid in the centre.

Weed seeds were treated together with the plant pathogens in laboratory batch digesters for one or two weeks (cf. above). In the two-stage digestion pilot plant, the bags were exposed to hydrolytic conditions for 9, 19 and 20 days, respectively. In the leach-bed (solid phase) digester, where mainly hydrolysis takes place, the bags were kept on three different levels: bottom, middle and top related to packing level. At the end of the digestion period, a selection of 2 x 20 seeds was grown on a normal available soil (Triohum, Samen Mauser, Dübendorf Switzerland). After a growing period of 20 days, the germination rate was evaluated (Kehres, 1989).

Laboratory Batch Digesters

The laboratory digestion experiments were carried out in 2 l wide-mouth bottles of polyethylene with a working volume of 1.5 l. The bottles were closed with rubber plugs, equipped with gas-tubes which were connected to gasometers. The biogas produced was collected in hydraulic gasometers. During the experiment, the bottles were placed in a water bath where constant digestion temperatures of 35 °C or 55 °C were maintained.

Two Stage Pilot Plant

The two stage pilot plant (Fig. 1) consisted of a Leach-Bed Solid Phase Digester (LBSPD) (hydrolytic step) and a Pulsating Dynamic Anaerobic Filter (Edelmann, 1989). The pilot digesters were equipped with external heat exchangers to maintain digestion temperature within the desired mesophilic range (app. 35°C).

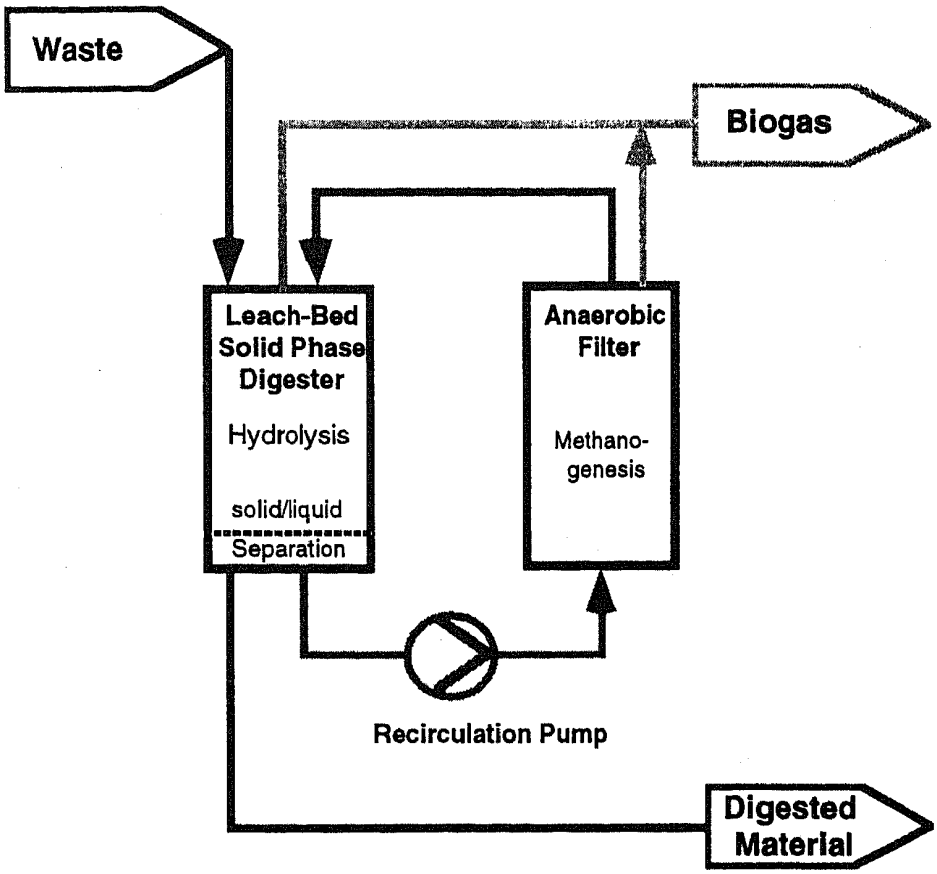


Fig. 1. Flow sheet of the two stage pilot plant

## RESULTS AND DISCUSSION

### Survival of Pathogens

The ability of root galls to infect bait plants was not reduced in anaerobic digestion at 35 °C but only at 55 °C (Table 1). A treatment of two weeks was necessary to obtain consistent results. Galls taken out from charges with 10% infested roots produced almost no clubroot on bait plants. When the amount of infested roots increased to 70% per charge the efficacy of the digestion was slightly reduced (Table 1, treatment 15). The most successful treatment showed an inoculum density of pathogens lower than the sensitivity of the test system, which corresponds to a 1000 fold inoculum reduction (data not shown). This degree of reduction will certainly be adequate for agricultural use of the end product, because the concentration of the pathogens is normally significantly lower than in the experiments described. The output of the digester will additionally be submitted to a short aerobic post-treatment, depending on use, thus further reducing pathogens. Furthermore the compost will be diluted when spread over the field. Nevertheless, regular checking of this anaerobic compost for plant pathogens is important. Especially before it is used in endangered cultures to guarantee maximum security to the growers.

TABLE 1. Influence of Anaerobic Digestion on the Survival of *Plasmodiophora brassicae*, Pathogenic Agent of the Clubroot Disease of Crucifers, in Cabbage Roots.

| Digester | Digestion | | | % diseased plants [a] | |
|---|---|---|---|---|---|
| | % infested roots per charge [b] | Temp. °C | Days | clubroot not digested | clubroot digested |
| D6 | 10 | 35 | 14 | 93.2 | 96.2 |
| D7 | 10 | 35 | 14 | 97.6 | 98.1 |
| D8 | 10 | 35 | 14 | 99.4 | 98.1 |
| D9 | 10 | 55 | 7 | 91.6 | 0.0 |
| D10 | 10 | 55 | 7 | 100.0 | 21.8 |
| D11 | 10 | 55 | 7 | 98.3 | 0.9 |
| D12 | 10 | 55 | 14 | 100.0 | 1.0 |
| D13 | 10 | 55 | 14 | 100.0 | 0.0 |
| D14 | 10 | 55 | 14 | 99.1 | 0.0 |
| D15 | 70 | 55 | 14 | 97.2 | 11.8 |

a % diseased china cabbage, which was observed after sowing 10% infested cabbage roots into 90% organic soil (6 pots with 25 - 35 seeds each per treatment )

b Digester charge: 10% infested roots, 60% bio waste and 30% digester inoculum, except D15:70% infested roots and 30% digester inoculum. Total weight of charge: 1.5 kg

## Survival of Weed Seeds

Anaerobic digestion of weed seeds at 55 °C during 7 and 14 days under laboratory conditions did completely eliminate germination of *Rumex* and *Lycopersicon lycopersicum* seeds (Table 2. D9 - D15). At 35 °C and a digestion period of 14 days, D7 shows a germination rate of 56% for *Lycopersicon lycopersicum,* whereas no germination was observed for D6 and D8. This might be due to the fact that the nylon bag was not in a close contact with the substrate in the batch digester D7.

To achieve a complete destruction of seeds during anaerobic digestion it seems to be recommendable to maintain a temperature of 55 °C for 14 days. This correlates with data and experience obtained while composting.

TABLE 2. Influence of Anaerobic Digestion on the Survival of *Rumex obtusifolius* and *Lycopersicon lycopersicum* (tomato) in Laboratory Batch Digesters

| Digester | | | *Rumex obtusifolius* [a] | | *Lycopersicon l.*[b] | |
|---|---|---|---|---|---|---|
| | | | Germ. rate | | Germ. rate | |
| | Temp.[°C] | Days | % absol. | % rel. | % absol. | % rel. |
| D6 | 35 | 14 | 0 | 0 | 0 | 0 |
| D7 | 35 | 14 | [c] | - | 48 | 56 |
| D8 | 35 | 14 | 0 | 0 | - | - |
| D9 | 55 | 7 | 0 | 0 | 0 | 0 |
| D10 | 55 | 7 | 0 | 0 | 0 | 0 |
| D11 | 55 | 7 | 0 | 0 | 0 | 0 |
| D12 | 55 | 14 | 0 | 0 | 0 | 0 |
| D13 | 55 | 14 | 0 | 0 | 0 | 0 |
| D14 | 55 | 14 | 0 | 0 | 0 | 0 |
| D15 | 55 | 14 | 0 | 0 | - | - |
| Untreated (Control) | | | 78 | 100 | 84 | 100 |

[a] relative germination rate of untreated *Rumex* seeds was 78%

[b] relative germination rate of untreated *Lycopersicon lycopersicum* seeds was 84%

[c] nylon bag was broken during digestion: not evaluateable

*Rumex* and *Lycopersicon lycopersicum* seeds exposed to hydrolytic conditions for 20 days at approx. 35 °C and a low pH of 5.6 to 7.6 were completely destroyed when placed in the leach-bed solid phase batch digester of a two stage pilot plant

This corresponds with results obtained (probag/arbi, 1990) in former studies, thus showing that hydrolytic conditions are mainly responsible for the killing of weed seeds during anaerobic digestion. This is not surprising, because hydrolytic enzymes, which are attacking cellulosic materials, are present in high concentration. These enzymes seem to be capable of destroying weed seeds in acidic conditions.
The data of Table 3 indicate that it is the anaerobic conditions prevailing in the filter and hydrolysis that are responsible for the elimination of weed seeds, but mainly the physical and (bio)chemical conditions within the hydrolysis.

TABLE 3. Influence of Anaerobic Digestion on the Survival of *Rumex obtusifolius, Digitaria sanguinalis* and *Lycopersicon lycopersicum* (tomato) in the Leach-Bed Solid Phase Batch Digester and in the Pulsating Dynamic Anaerobic Filter at Different Exposure Times

| Test Site/ Digestion time Seeds | Germ. rate [a] % absol. | Germ. rate % rel. |
|---|---|---|
| Hydrolysis 9 Days | | |
| *Rumex o.* | 31.00 | 57.90 |
| *Digitaria s.* | 0.00 | 0.00 |
| Hydrolysis 19 Days | | |
| *Rumex* | 4.40 | 13.40 |
| *Digitaria* | 0.00 | 0.00 |
| *Lycopersicon l.* | 0.00 | 0.00 |
| Filter 19 Days | | |
| *Rumex o.* | 33.60 | 62.90 |
| *Digitaria s.* | 0.30 | 3.90 |
| Untreated (Control) | | |
| *Rumex o.* | 53.50 | 100.00 |
| *Digitaria s.* | 8.50 | 100.00 |
| *Lycopersicon l.* | 74.40 | 100.00 |

[a] # of seeds per test: 200 to 400

In the studies mentioned above (probag/arbi 1990) the product qualities of digested materials were also compared. The substrates originated from AN, BTA, DRANCO and VALORGA. The digested substrates of AN, BTA and DRANCO showed higher yields during cress growth tests than control (commercially earth) and composts (after 7 days of controlled growth more than 10 g of cress fresh weight per g of cress seeds; growth on 100% digested substrate). The substrate of VALORGA showed reduced growth (6.7 g fresh weight /g seeds). Tests on the germination and the growth of the roots of beans (Phaseolus vulgaris) showed a significantly reduced germination on VALORGA substrate compared to other substrates. One might suppose that the reduced growth on VALORGA substrate was a consequence of the chemical qualities of the organic waste, which - in comparison to other substrates - was not collected separately , but only freed from metals and other fractions before digestion. All substrates tested according to the method of Kehres et al. (1989) showed negligible contents of viable weed seeds (less than 1 germ per litre of substrate).

## CONCLUSIONS

More detailed studies have to be made with laboratory digesters to obtain further information about the factors influencing pathogen destruction. As a next step, the survival ability of pathogens has to be tested under full-scale conditions. Furthermore, the survival ability of some other very heat- and composting-resistant fungi should also be tested. Especially *Pyrenochaeta lycopersici*, causing corky-root on tomatoes, which is known to be even more resistant to composting than *Plasmodiophora brassicae*. Facing the growing amounts of wastes which have to be treated, it is very important to be able to certify composts for the absence of pathogens in a future market.

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