Quality Assessment of Biogas Plant End Products by Plant Bioassays

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Abstract—**Biogas technology offers a sustainable process for production of renewable energy, management of biodegradable waste, and production of nutrient-rich digestate products for fertilizing and soil improvement purposes. In order to promote the utilization of such digestate products effectively, we need more information on their quality, safety, and applicability in crop production. We have used plant bioassays to evaluate the quality and potential phytotoxicity of various digestate products obtained from three full-scale biogas plants processing organic waste and by-products from agriculture, industry and municipalities in Finland. Germination, growth and health of three test plants, including barley, Chinese cabbage, and cress, were monitored in the presence of digestate products in concentrations used in field conditions. The results suggest that both solid and liquid end products from biogas plants can be utilized as fertilizers or soil improvers for production of cereal crops such as barley.**

Index Terms—**Biogas plant, bioassay, digestate, phytotoxicity, quality.**

I. Introduction

In the future, mankind will be challenged by an increase in human population at the same time as the inputs required for food production including arable land, inorganic minerals, and energy based on fossil fuels are decreasing. On the other hand, generation of waste is still increasing in proportion to income, along with the economic and environmental costs associated with expanding landfills.

Biogas technology offers a competitive process to manage biodegradable waste streams and to produce renewable energy in a sustainable way. Furthermore, the nutrients present in the waste materials are preserved in the anaerobic digestate, which can be further refined into value-added products, such as organic fertilizers and soil improvers [1]. Utilization of biogas plant (BGP) end products in crop production promotes the recycling and conservation of organic matter and minerals such as phosphorus, and reduces the need for energy-intensive nitrogen fertilizers.

Large-scale utilization of BGP end products in crop production necessitates safety assessment studies and proof of product applicability for intended use. In Finland, the quality of organic materials intended for soil fertilizing and improvement is regulated by legislation and the decree of the Ministry of Agriculture and Forestry aiming at safe fertilizer products. Several criteria must be taken into account when considering possible adverse effects of the products on plants (i.e. phytotoxicity) or the environment. These include the maturity and stability of organic material, the reduction of plant and animal pathogens, and the absence of harmful compounds such as heavy metals and persistent organic pollutants.

Both the producers and supervisory authorities need standardized methods and quality criteria in order to monitor the product quality and safety requirements set by law. The tests intended for routine control should be rapid, sensitive, and economical. Whereas chemical methods can be used to determine the chemical composition of the products, the data often remains insufficient and fails to predict the overall effects on exposed plants and the soil ecosystem [2]. Bioassays overcome these limitations by directly exposing living organisms, such as microbes [3], plants [4], [5] [6] or soil invertebrates [2], [4], [6] to the products under test. Bioassays include possible interactions between various chemical compounds and the complex matrix and provide more realistic information on the product quality. In the present study, we have used plant bioassays to evaluate the quality of various end products from three different biogas plants processing organic waste and by-products from agriculture, industry and municipalities in Finland. Furthermore, we evaluated the suitability of the methods for routine quality monitoring by the producers and supervisory authorities.

The BGP end products tested in concentrations used in the field conditions showed no significant phytotoxicity for barley in comparison to manure, suggesting that they are applicable as fertilizers or soil improvers for production of cereal crops. The simple and sensitive cress germination test on a Petri dish proved to be suitable for routine quality analysis of BGP end products.

The work described here is part of a larger project "Processing biogas plant digestates into value-added products – BIOVIRTA" [1], which aims to develop technologies and practices to refine digestates into competitive and safe products for various end uses.

II. Experimental Study

A. Samples

Three types of samples (anaerobic digestate, and separated solid and liquid fractions of the digestate) were collected from three full-scale biogas plants in Finland on three separate occasions during 2010. All BGPs used mesophilic anaerobic digestion to process organic waste and by-products from agriculture, industry and municipalities, but their hygienization processes differed from each other (Table 1). Pig manure and inorganic fertilizer were used as reference samples.

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TABLE I: SAMPLES ANALYZED IN THIS STUDY

Sample origin	Raw material	Hygienization process	Sample type
BGP 1	Pig manure (90%), industrial by-products (10%)	1 hr, 70 °C (pre-digestion)	Digestate 1, Solid 1, Liquid 1
BGP 2	Sewage sludge	30 min, 150-160 °C and 5 bar (pre-digestion)	Digestate 2, Solid 2, Liquid 2
BGP 3	Source-separated biowaste (20%), sewage sludge (70%), grease trap sludge (10%)	1 hr, 80°C (post-digestion)	Digestate 3, Solid 3, Liquid 3
Controls			Artificial standard soil, sphagnum peat, commercial growing medium
References			Pig manure, inorganic fertilizer (NPK)

B. Sample Preparation

As the BGP end products are intended to be used as fertilizers or additives rather than growth medium *per se*, samples from BGPs were diluted prior to testing with artificial soil [7], sphagnum peat [8], or a peat-based commercial growing medium (CGM) in a ratio corresponding to their actual use in the field. The mixing ratio was determined by the ammonium nitrogen content of the samples according to the requirements for agricultural environmental aid [9]. The target value was set at 90 kg NH_4-N/ha. The concentration of ammonium nitrogen was determined by using the Kjeldahl method (method 984.13) and a Foss Kjeltec 2300 Analyzer Unit (Foss Tecator AB, Höganäs, Sweden). Cu was used as the digestion catalyst. The mixtures were prepared on volume/volume basis of the laboratory bulk densities of their constituents, measured according to [10].

The mixtures were supplemented with commercial trace nutrient mixture, and their pH was adjusted with ground limestone to 5.5 – 6.0 for sphagnum peat [8], and 6.0 – 6.5 for artificial soil [7]. Adjustment of pH is critical since it is known to affect the germination and primary root growth of plants and therefore must be taken into account when analyzing the results of bioassays. Prior to sample analysis, optimal water content of the samples and controls was adjusted using the “fist test” [8].

C. Pot Growth Tests

The plant bioassays used to evaluate the quality of BGP end products are shown in Table 2.

In pot growth tests, modified from [8], ten seeds of Chinese cabbage (*Brassica pekinensis*) or barley (*Hordeum vulgare*) were sown in each of five replicate pots (374 ml, 10 cm diameter) containing the diluted end products from BGPs. The plants were grown under controlled conditions (6000 lux, 16 hrs per day/8 hrs per night, 30°C/18°C, and 40-60% humidity). The number of germinated seeds was calculated after five days and the fresh weight of shoots (cut at the substrate level) was determined after 18 days of growth. Inhibition of germination (GeI, %) and inhibition of growth (GrI, %) were calculated according to [8] using (1) and (2), respectively.

$$\text{GeI (\%)} = \frac{\text{AGR control - AGR sample}}{\text{AGR control}} \times 100 \quad (1)$$

$$\text{GrI (\%)} = \frac{\text{APW control - APW sample}}{\text{APW control}} \times 100 \quad (2)$$

where
AGR is the average germination rate
APW is the average plant weight

The average chlorophyll content of the leaves was measured using a Spad-502 chlorophyll meter (Konica Minolta, Japan). The relative change in the color of plants grown in the presence of BGP end products was calculated using (3).

$$\text{CI (\%)} = \frac{\text{AC control - AC sample}}{\text{AC control}} \times 100 \quad (3)$$

where AC is the average leaf color (n=5)

The morphology and length of the barley roots were inspected visually after 18 days of growth in diluted BGP end products or in control substrates. The roots were washed carefully, measured, and photographed.

D. Petri Dish Tests Using Cress

Phytotoxicity assays were performed by germinating ten cress seeds (*Lepidium sativum*) in direct contact with each diluted sample on three replicate (100 mm x 100 mm) Petri dishes [11]. The Petri dishes were placed in a growth chamber 70-80° to the horizontal and incubated at 25°C in darkness for 72 hours. Thereafter, the number of germinated seeds and length of primary roots were measured.

The Munoo-Liisa vitality index (MLV, %), which takes into account both seed germination and growth of primary roots, was calculated according to [11] using (4).

$$\text{MLV(\%)} = \frac{(\text{GRs1}\times\text{RLs1}) + (\text{GRs2}\times\text{RLs2}) + (\text{GRs3}\times\text{RLs3})}{(3\times\text{GRc}\times\text{RLc})} \times 100 \quad (4)$$

where
GR is the germination rate
RL is the average root length
c is the control
s1, s2, and s3 are the first, second, and third sample replicate, respectively

TABLE II: PLANT BIOASSAYS USED IN THIS STUDY

Method	Description
Pot growth test, modified from [8]	Cultivation of Chinese cabbage and barley in the sample under controlled conditions for 18 d. Measured parameters: germination, fresh weight and leaf color relative to control.
Petri dish test using cress [11]	Cultivation of cress seeds in the sample for 72 hrs in the dark. Measured parameters: germination and primary root length relative to control.

TABLE III: GERMINATION INHIBITION OF CHINESE CABBAGE AND BARLEY IN THE PRESENCE OF BGP END PRODUCTS.

Plant	Diluent	Digest. 1	Digest. 2	Digest.3	Solid 1	Solid 2	Solid 3	Liquid 1	Liquid 2	Liquid 3	Manure	NPK
Chinese cabbage	Soil	-0.7 (3.9)	0.0 (0.0)	1.0 (1.4)	1.7 (2.4)	-1.7 (2.4)	8.9 (6.9)	-1.7 (2.4)	1.0 (1.4)	-0.7 (3.9)	2.0	4.0
Chinese cabbage	Peat	0.0 (0.0)	-1.7 (2.4)	12.1 (17.1)	1.3 (6.7)	2.0 (2.8)	0.0 (0.0)	2.3 (8.1)	7.2 (4.5)	3.4 (4.9)	2.0	0.0
Barley	Soil	-2.2 (2.0)	-2.9 (0.8)	1.8 (8.1)	-2.2 (2.0)	1.5 (2.4)	3.4 (5.7)	-1.4 (6.8)	1.8 (3.0)	1.5 (4.0)	-5.3	-1.1
Barley	Peat	-7.7 (10.9)	0.0 (0.0)	1.8 (8.4)	-3.8 (5.4)	7.0 (1.0)	9.8 (7.9)	-6.8 (6.7)	7.7 (10.9)	-5.8 (8.2)	0.0	2.1
Barley	CGM				-6.4	4.3	6.4					

The values are expressed as percentages of inhibition relative to respective controls. A negative value indicates an increase in germination relative to control. Standard deviations are given in parentheses.

III. RESULTS

The ammonium-nitrogen concentrations of the BGP end products varied depending on the composition of raw materials and the type of end product. The highest NH_4-N concentrations (5.9-6.0 g kg^{-1}) were observed in the digestate and liquid fractions of BGP1, whereas the NH4-N concentrations in the other BGP products ranged between 1.8-3.8 g kg-1 (data not shown). The higher NH4-N concentrations in the BGP1 end products as compared to other BGPs was apparently due to a high proportion of pig manure in the BGP1 input.

The average germination rate of Chinese cabbage and barley seeds in the presence of BGP end products was mostly over 90%. Slightly lower germination rates, ca. 80 - 90%, resulting in germination inhibition of 7.0 to 12.1%, were observed in four products, including the digestate and solid fractions of BGP3, and liquid and solid fractions of BGP2 (Table 3). Nevertheless, the germination rate of test plants in all diluted BGP end products can be considered good.

More variation was observed when the effect of BGP end products on plant growth was measured (Fig. 1). For barley, amendment of standard soil or sphagnum peat with various BGP end products generally increased the average fresh weight of shoots similarly to pig manure, which was used as a reference. However, there was a notable variation in results between replicate samples taken at different time points, and despite the average positive effect, certain individual samples exerted an inhibitory effect on barley growth. In commercial growing medium, the solid BGP end products had only a minor effect on barley growth. The growth of Chinese cabbage was generally retarded in the presence of BGP end products (Fig. 1). An interesting exception to this was the solid fraction of BGP3, which substantially stimulated the growth of Chinese cabbage both in standard soil and in sphagnum peat.

The liquid fraction of BGP3 also had only a minor negative effect on Chinese cabbage growth. Pig manure diluted with standard soil inhibited the growth of Chinese cabbage to a similar extent as the BGP end products, whereas manure diluted with peat had no significant effect on plant growth (Fig. 1).

The average chlorophyll content of Chinese cabbage leaves mostly correlated with the results of plant growth (Fig. 2). The chlorophyll content decreased or remained close to the control level in the presence of BGP end products, except for the liquid fraction of BGP2 in soil, which showed a slight positive effect on plant color. Manure diluted with either soil or peat also had a noticeable negative effect on Chinese cabbage color. The effects of BGP end products on barley shoot color were relatively minor. Similar to the growth experiments (Fig. 1), the variation in results between different sampling times was high.

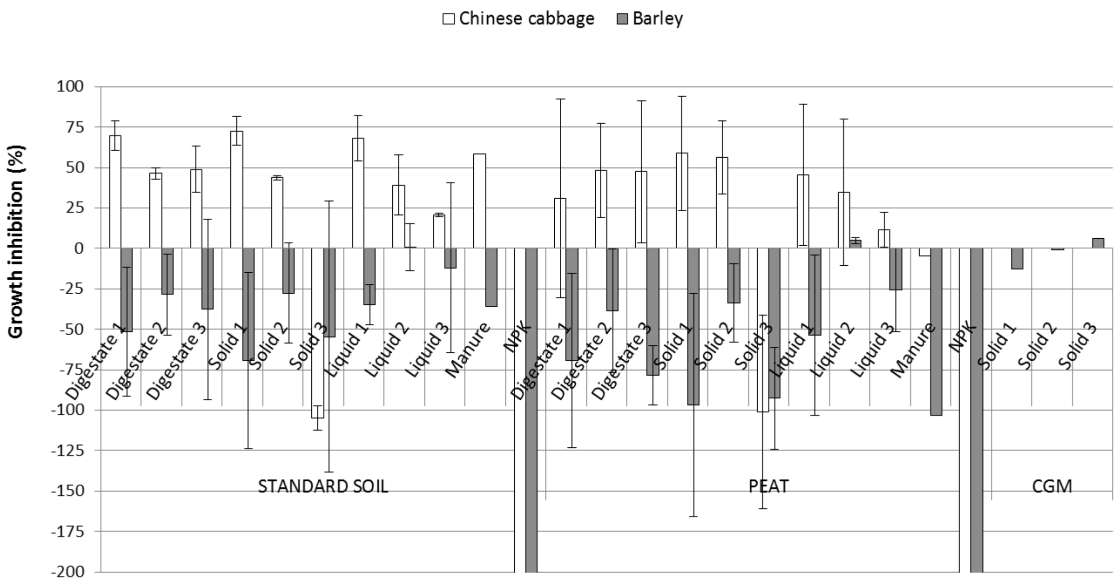

Fig.1. Growth inhibition of Chinese cabbage and barley seedlings in BGP end products diluted with soil, peat or CGM. Negative values indicate enhanced plant growth relative to respective controls.

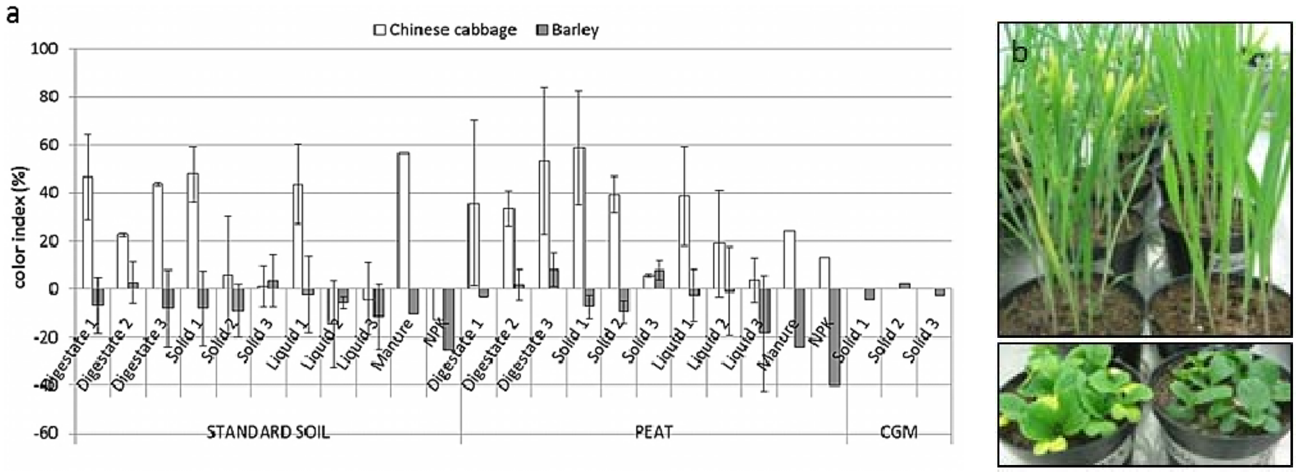

Fig. 2 (a) The effect of BGP end products on chlorophyll content of Chinese cabbage and barley leaves. The plants were grown in BGP end products diluted with soil, peat or CGM. The color index indicates the average chlorophyll concentration of plant leaves relative to the control. Positive index indicates a decrease in chlorophyll content. (b) Color differences in the leaves of barley and Chinese cabbage grown in various test substrates.

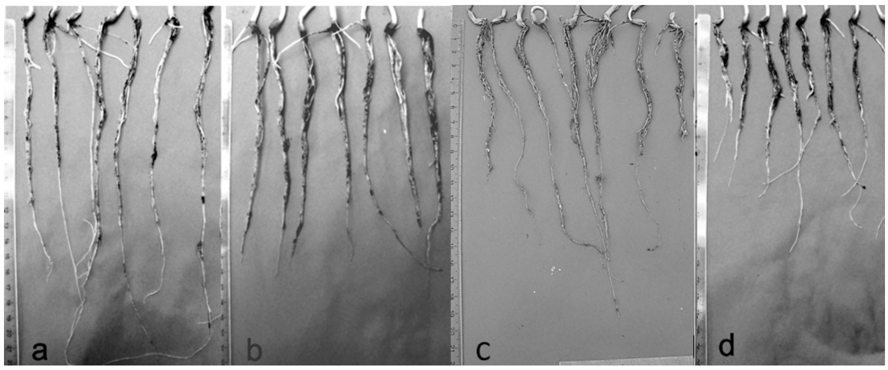

Fig. 3. Barley roots grown in (a) standard soil, (b) CGM, (c) soil-diluted liquid fraction from BGP1 and (d) soil-diluted solid fraction from BGP3.

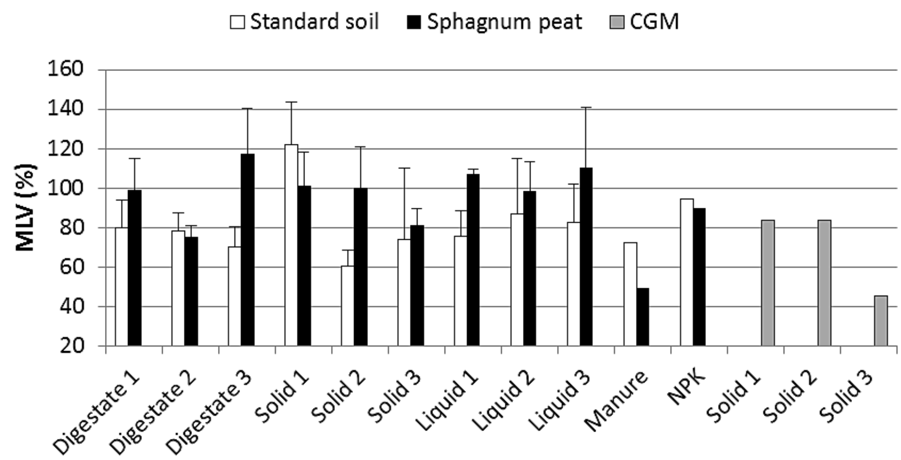

Fig. 4. Munoo-Liisa vitality index (MLV %) which compares germination of cress seeds in the tested material (%) and the mean root length in the test and control samples after 72 hours. The results are expressed relative to the control (100%).

Visual inspection of barley roots showed that root lengths in most BGP end products diluted with soil were comparable or slightly shorter than in soil and only two products, the liquid fraction of BGP1 and solid fraction of BGP3, noticeably inhibited root growth (Fig. 3). Cultivation of barley in CGM resulted in shorter roots as compared to those grown in standard soil. Amendment of CGM with solid BGP fractions had little effect on the length or density of the barley root system (data not shown).

The results of the cress germination test, expressed as Munoo-Liisa vitality index (Fig. 4), take into account both the germination rate and root elongation of cress seedlings. In general, the MLV indices in peat-diluted BGP end products were slightly higher than in soil or CGM-diluted end products. The MLV indices in peat-diluted BGP products also exceeded that of manure, and with the exception of two products (digestate of BGP2 and solid fraction of BGP3), also that obtained with mineral fertilizer. Amendment of standard soil with BGP end products inhibited cress growth slightly, except for the solid fraction of BGP1, which stimulated cress growth when compared to those grown in standard soil with mineral fertilizer addition. Nevertheless, the MLV values in soil-diluted BGP end products were comparable to, or even higher than those in manure.

IV. Discussion

According to Finnish legislation, organic fertilizer products must be safe and pose no harm to humans, animals or the environment. The mandatory criteria set by law aim at protecting the environment and consumers from negative effects caused by potential pathogens, toxic elements, weed seeds and other impurities in fertilizer products. In addition, quality assessment programs often include non-compulsory criteria including threshold values, which are related to various end uses of the products [12]. In products intended for agricultural use, lack of phytotoxicity is a particularly important quality factor, since it has a direct impact on crop yield and quality.

The simple cress germination test on a Petri dish appeared to be promising in predicting the potential phytotoxicity of BGP end products. In line with previous reports [13] root growth was a particularly sensitive indicator, whereas germination remained less informative due to the high germination rates of cress seeds in all samples. The target value for MLV % in cress germination tests has been set at 80% in the Finnish Act on Fertilizer Products, and values below that should be taken as an indication of potential phytotoxic compounds in the product. With the exception of BGP2 digestate, all BGP end products diluted with peat achieved the 80% target value. The MLV % of BGP end products diluted with standard soil were slightly lower, and only four out of nine products achieved the target value. However, manure, which is a conventionally used fertilizer in the fields, also remained below the target value both in soil (MLV 72%) and peat (MLV 49%) mixtures. In practice, therefore, the 80% target value may be too high, and MLV indices above 60% should be considered adequate for good product quality. Since the MLV indices obtained with BGP end products were comparable or higher than those of manure, they can be considered fit for agricultural use and fit for use as fertilizers or soil improvers in agriculture.

Moreover, the germination rate and root growth of cress seeds were higher in peat amended with BGP end products than in peat fertilized with NPK. This suggests that the BGP end products offer additional advantages to plant growth that cannot be obtained by using mineral fertilizer alone. In addition to nutrient effects, organic material has been shown to have positive effects on soil composition through stimulation of microbial activity and root growth [14], [15], therefore promoting soil health and plant growth better than inorganic fertilizers.

Pot growth tests with Chinese cabbage and barley showed minor differences in seed germination with either test plant in contrast to shoot fresh weight determination, which can be considered as an applicable quality criteria. The pot growth test with barley showed that the vast majority of BGP end products studied promoted the growth of barley relative to controls and, in some cases, the growth stimulation was similar to or even better than that obtained with pig manure. A positive impact by BGP end products on barley color may also be deduced, although the changes relative to control were minor. With the exception of two products, no significant inhibition of root growth by BGP end products was observed. Taken together, the results support the view that BGP end products can be utilized as fertilizers in the production of cereal crops such as barley.

The pot growth test with Chinese cabbage demonstrated a significant growth inhibition in all but one product, namely the solid fraction of BGP3, when compared to control and manure. Furthermore, the general decrease in chlorophyll content suggested that the plants were under stress. The pot growth test clearly demonstrated the differences in intrinsic sensitivity and nutrient demand of barley and Chinese cabbage. Chinese cabbage proved to be a more sensitive test plant than barley, and can thereby depict smaller variations in the quality of BGP end products. In fact, Hordeum vulgare is considered to be one of the most tolerant cereal crops, and has been shown to tolerate high amounts of salt [16], [17] and heavy metals [18], for example. In line with our results, barley has been shown to be a less sensitive test plant than, for example, radish, cress or lettuce in laboratory assays [19], [20], [21].

Interpretation of phytotoxicity based on plant bioassays is challenging. The results varied depending on the plant species, control substrate (soil, peat or CGM) and the test protocol used. In line with our results, other research groups have reported plant species–dependent differences in sensitivity to various chemical compounds [20], [21], [22]. This stresses the importance of using several different plant species in phytotoxicity experiments, even though the intended purpose for using the product would be on the barley field.

Variation in the results emphasizes the importance of using appropriate controls, especially when dealing with heterogeneous biological materials. In general, the germination and root growth of cress was better in peat-diluted BGP end products as compared to those diluted with standard soil (Fig. 4). Similarly, growth of barley was more pronounced in BGP end products diluted with peat in the pot growth test (Fig. 1). There was, however, a large variation in the results between different sampling times due to the heterogeneous feedstock at BGPs during 2010. In order to ensure that the variation originates from the test materials only, it is essential to have uniform and reproducible controls.

Optimally, control soil should have similar physico-chemical characteristics to the soil under study, since factors such as pH, electric conductivity, and water holding capacity can have a significant impact on the results. In practice, however, aspects such as easy preparation and readily available ingredients often influence the choice of control substrates. The tests intended for routine quality monitoring of organic fertilizer products should be sensitive, ecologically relevant, simple, and economical. The pot growth test with barley, despite its agronomic relevance, may be too insensitive due to the high tolerance and adaptability of barley to the presence of various harmful compounds [21]. Measuring and visually inspecting the growth of barley roots in peat-based substrates, on the other hand, is laborious and time consuming and therefore cannot be recommended for routine analysis of this type of sample. The cress germination test on a Petri dish turned out to be both sensitive enough to detect variation in the quality of BGP products, and simple enough to serve as a feasible test in quality monitoring requiring rapid throughput times. A similar kind of soil plate assay utilizing cress, lettuce, and barley, was chosen as the most sensitive and feasible plant assay for studying the phytotoxicity of heavy metals in soils [21].

Taking the factors discussed above into account, plant bioassays can be applied for the quality assessment of BGP end products. However, in order to interpret results in a reliable way, it is essential to understand the chemical and physical characteristics of the test and control materials. In general, evaluation of the quality of organic fertilizer products requires several methods, both chemical and biological [23], [24] or the use of a test battery composed of several complementary assays [25], [19] in order to avoid false results.

V. Conclusion

This project has provided new information on the quality and quality assessment methods of BGP end products, thereby enhancing the operational preconditions for biogas plants. Plant bioassays showed that the BGP end products tested in concentrations used in field conditions promoted the growth of barley similarly to or even better than manure, which is a conventionally used fertilizer in the fields. The results support the view that both solid and liquid end products of BGPs can be utilized as fertilizers or soil improvers for cereal crops such as barley. Among the plant bioassays, the cress germination test on a Petri dish with its simplicity and sensitivity proved to be the most promising assay for routine quality monitoring of organic fertilizer products.

Future studies include comparison of these laboratory pot growth results to those from field trials using the same BGP end products. Additionally, contents of organic pollutants, pharmaceuticals and hormones and the possible accumulation of these chemicals into the soil and food chain will be studied.

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