Annex 5:

Preliminary Results from the Soil Incubation Study/Pot Experiment on Fertilizer Value of Anaerobically Digested Slurries from a Co-fermentation with *Ulva lactuca*

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Preliminary Results from the Soil Incubation Study/Pot Experiment on Fertilizer Value of Anaerobically Digested Slurries from a Co-fermentation with *Ulva lactuca*

Objectives of the Study

The objectives of the study were:

1) to determine the fertilizer value of the effluents originating from cattle manure co-digested with *Ulva lactuca* in comparison to the anaerobically digested cattle slurry alone

2) to investigate the potential greenhouse gas emissions (N_2O, CO_2) after application of the different slurries and

3) to obtain information about key soil processes underlying the observed effects.

To achieve these aims, a pot experiment with barley plants and a soil incubation study were set up simultaneously. The incubation study was used to intensively follow the evolution of greenhouse gases and key soil processes during the first three weeks after incorporation of the different soil amendments, while the pot experiment was meant to give indication of the longer-term plant response to the effluents.

Materials and Methods

Soil Used

The soil selected for the incubation study and the pot experiment was taken from the 0-25 cm layer of an agricultural field at Risø DTU (55°41'N, 12° 05'E) with 11% clay, 14% silt, 49% fine sand, and 25% coarse sand (Typic Hapludalf). The soil was air-dried and sieved to obtain the fraction ≤ 1 cm.

Incubation Study

The experiment was carried out using 200 g dry soil, incubated in a 330 ml PVC container. Before the start of the incubation, the soil was wetted and pre-incubated at room temperature for 14 days.

Seven different substrate treatments have been set up:
Soil 200 g (CO)
Soil 200g + mineral fertilizer (MIN)
Soil 200g + wet *Ulva lactuca* (UW)
Soil 200g + dried and ground *Ulva lactuca* (UD)
Soil 200 g + biogas effluent from cattle manure (SL)
Soil 200 g + biogas effluent from co-digested cattle manure with *Ulva lactuca* (20%, SL UL)
Soil 200 g + biogas effluent from co-digested cattle manure with *Ulva lactuca* (40%, SL UH)

The different amendments were added at rates to provide 30 mg total N per kg dry soil and mixed thoroughly into the soil. The mineral fertilizer was added in a solution of ammoniumnitrate and potassiumdihydrogenphosphate (20 mg P/kg dry soil and 30 mg K/kg dry soil) as well as magnesiumsulfate (20 mg Mg/kg dry soil). The amount of water added was adjusted to reach a WFPS of 65%.
Each individual container was sealed with a pierced lid, and the soil moisture content was kept constant during the experiment. The containers were incubated at constant 21°C in a thermo cabinet throughout the 23 days experiment**.**

Pot Experiment
The pot experiment comprised the same treatments as the incubation study. All amendments were thoroughly mixed to 2 kg soil (dry weight basis) which was then filled in black plastic pots (height 17 cm, diam. 15 cm, volume 3 l). Eight spring barley seeds (cv. Power) were sown per pot and plants were thinned to four per pot after emergence. The pots were set in a growth chamber with 21°C/16°C day/night and a 16 h day length and irrigated as required.

Greenhouse Gas Analyses
Carbon dioxide and N_2O emissions were measured on days 2, 3, 4, 5, 8, 10, 12, 15, 18, and 22 during the incubation. The container lids were opened and each container was placed in a 2 l gas-tight glass jar. Gas accumulation in the headspace was measured using a Photoacoustic Field Gas-Monitor (INNOVA 1412 Photoacoustic Field Gas Monitor,

LumaSense Technologies, Ballerup, DK) equipped with three individual optical filters (CO2 , N2O and water vapor), connected in a closed loop to the glass jar via two valves mounted in the lid and side of the jar, respectively. Headspace concentrations were measured approximately 0, 30, 60, and 90 min. after closing the jars and flux rates were calculated using linear regression. Linearity of emissions was always tested and only measurements with statistically significant regressions or an $R^2 \geq 0.95$ were taken into account.

Soil Analyses

Destructive soil samplings were performed on days 1, 8, 23 for the incubation study and day 1, 23 and 59 for the pot experiment. Ten g of soil sample were extracted with 50 ml 0.5 M K_2SO_4 for 60 min and the extracts were filtered through pleated filters (Grade 74, Frisenette Aps, Denmark). Concentrations of ammonium (NH_4+) and nitrate (NO_3-) were determined colorimetrically. Dissolved organic carbon (DOC) was extracted by the same procedure and analyzed on a TOC-VCPH (Shimadzu).

Another set of subsamples (10g) was vacuum-incubated with chloroform for 24 before extraction to determine microbial biomass C and N (Vance, et al., 1987). The biomass C was measured from the relationship $C_{biomass}$=2.22 x EC, where EC is $[DOC_{fumigated}]$-$[DOC_{unfumigated}]$ (Wu, et al., 1990). The biomass N was measured from the relationship $N_{biomass}$=1,85 x EN, where EN is $[TDN_{fumigated}]$-$[TDN_{unfumigated}]$ (Joergensen and Mueller, 1996). Soil pH was determined in a 5:25 (w/v) suspension of fresh soil in distilled water. 10 g of fresh soil were extracted with 50 ml Milli-Q water (agitated 60 min) followed by filtration. The organic carbon pool measured in the extract, using a Shimadzu TOC-VCPH analyzer (Shimadzu Corp., Kyoto, Japan), is referred to as cold-water-extractable carbon (CWEC).

Plant-available phosphorus (Olsen-P) was measured after extraction of 2 g fresh soil with 30 ml $NaHCO_3$ (pH 8.5) by standard colorimetric procedures on an AutoAnalyzer 3 (Bran+Luebbe, Norderstedt, Germany).

Plant Analyses

Plant dry matter per pot was recorded twice, after three weeks (together with the last destructive soil sampling of the incubation containers) and at the end of the experiment.

The dried material was ground and analysed for its total N content using an EA-1110 CHN elemental analyzer (CE instruments, Milan, Italy). The P uptake was calculated after wet digestion of the plant material and analysis of the P content as described above.

Preliminary Results

Plant Response in the Pot Experiments

The plant dry matter recorded was very similar among all treatments at both harvest dates (21 and 59 days after sowing, Figure 1). Only the treatment receiving mineral fertilizer resulted in a significantly increased dry matter production, whereas all other soil amendments only caused a slight, insignificant increase in dry matter compared to the untreated control.

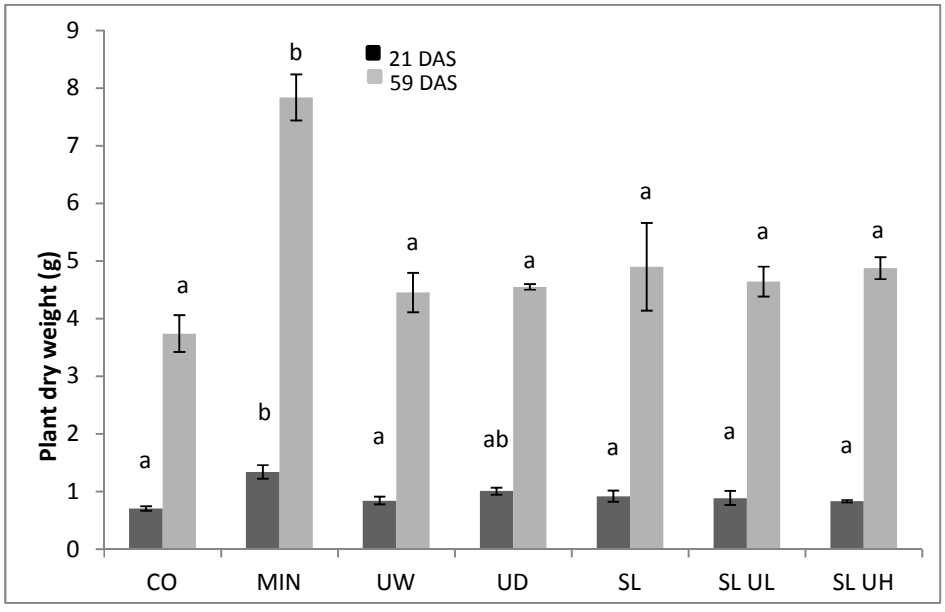

Figure 1: Plant dry weight in the pot experiment at two different harvest dates. DAS= days after sowing. CO=non-amended control soil, MIN= soil + mineral fertilizer, UW = soil+wet *U. lactuca, UD*=Soil+dried and ground *U. lactuca*, SL= soil+biogas effluent from cattle manure, SL UL= Soil+biogas effluent from co-digested cattle manure with *U. lactuca* (20%); SL UH=Soil+biogas effluent from co-digested cattle manure with *U. lactuca* (40%). Bars with different letters within the same sampling dates are not significantly different at p≤0,05.

Plant Nutrients in Soil in Incubation and Pot Experiment

The mineral N concentration in the soil at the beginning of the experiment was similar in both the minerally fertilized treatment and in the treatment receiving digested cattle manure (Figure 2).

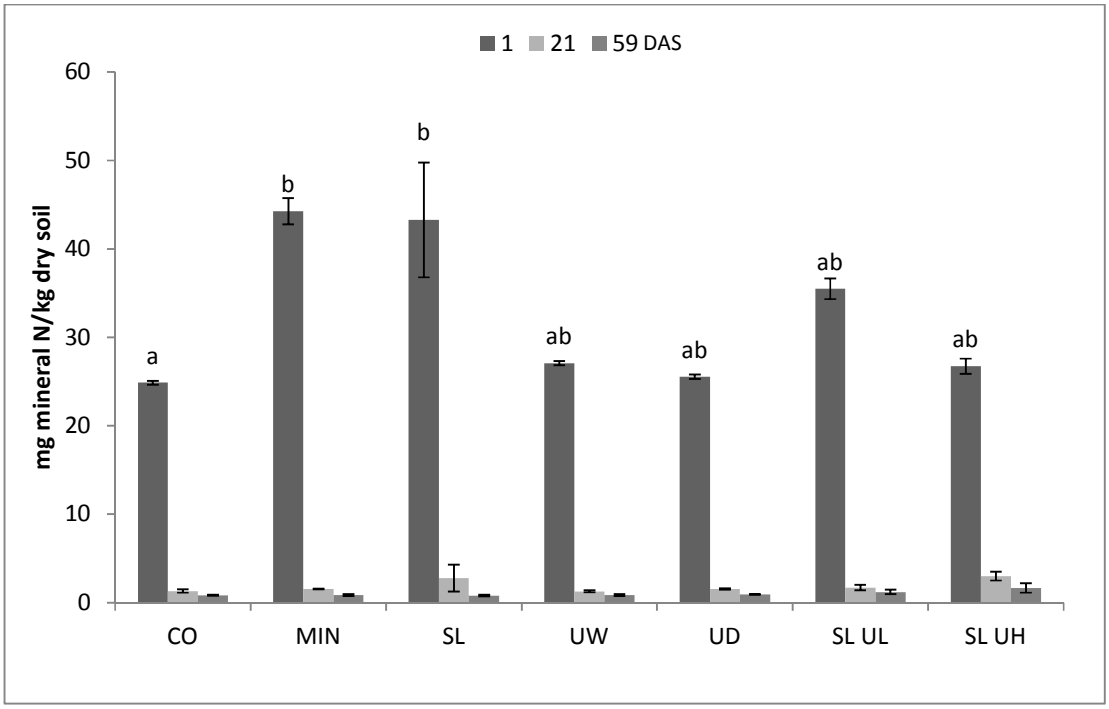

Figure 2: Concentration of soil mineral N in the pot experiment at different sampling dates. DAS= days after sowing. CO=non-amended control soil, MIN= soil + mineral fertilizer, UW = soil+wet *U. lactuca, UD*=Soil+dried and ground *U. lactuca*, SL= soil+biogas effluent from cattle manure, SL UL= Soil+biogas effluent from co-digested cattle manure with *U. lactuca* (20%); SL UH=Soil+biogas effluent from co-digested cattle manure with *U. lactuca* (40%). Bars with different letters are not significantly different at p≤0,05.

The treatments that received slurry from co-digestion with *Ulva* showed lower levels of mineral N at the beginning of the experiment especially when the proportion of *Ulva* in the slurry was high. However, in the soil incubation experiment similar levels of mineral N in all slurry treatments (36-39 mg/kg dry soil) were detected after 8 days, indicating a high N mineralization potential from the slurries containing *Ulva*. At the first harvesting date, mineral N concentration in soil was already very low in all pots, being only marginally higher in the slurry treatments. The differences in plant dry matter between CO and the *Ulva* treatments on the one side and the minerally fertilized treatment on the other could therefore be well

explained by initial differences in soil mineral N content. However, the observed differences in dry matter between the slurry treatments and the minerally fertilized treatment are not as obvious to interpret, but will probably be clarified after determination of the plants' N uptake.

Plant-available phosphorus in the soil may have been another factor limiting plant growth in the organically fertilized treatments (Figure 3), since initial Olsen-P was only increased in the minerally fertilized treatments compared to the non-treated control. A decrease of plant-available phosphorus (probably by plant-uptake) during the first three weeks could be observed in all treatments, but was apparently much greater in the MIN treatment than in the organically amended soils.

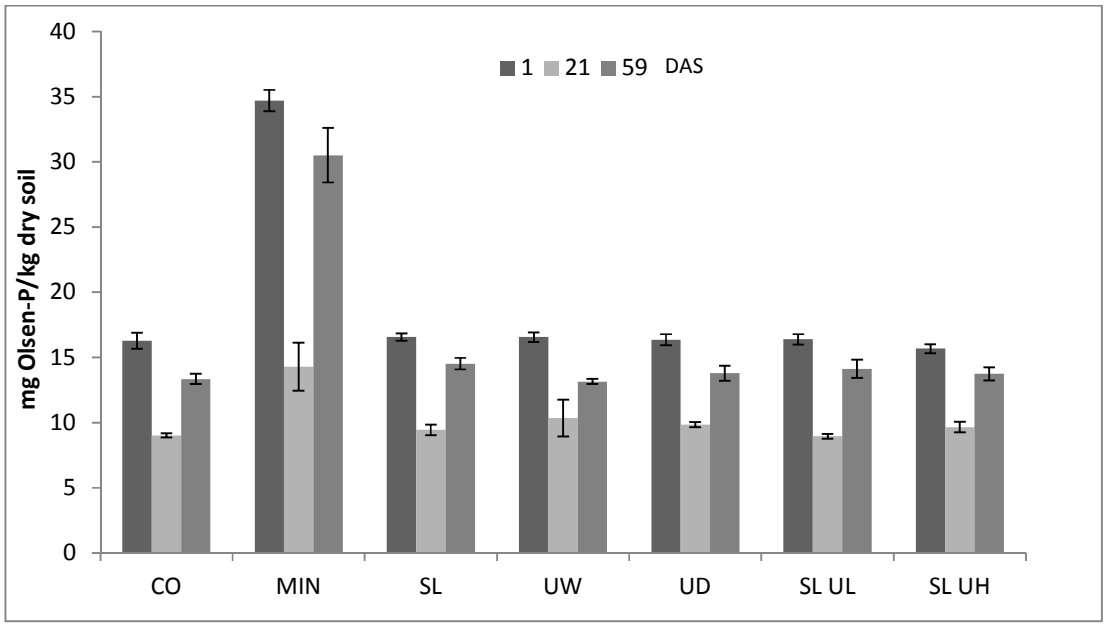

Figure 3: Concentration of Olsen-P in the pot experiment at different sampling dates. DAS= days after sowing. CO=non-amended control soil, MIN= soil + mineral fertilizer, UW = soil+wet *U. lactuca, UD*=Soil+dried and ground *U. lactuca*, SL= soil+biogas effluent from cattle manure, SL UL= Soil+biogas effluent from co-digested cattle manure with *U. lactuca* (20%); SL UH=Soil+biogas effluent from co-digested cattle manure with *U. lactuca* (40%).

Greenhouse Gas Emissions in the Soil Incubation Study

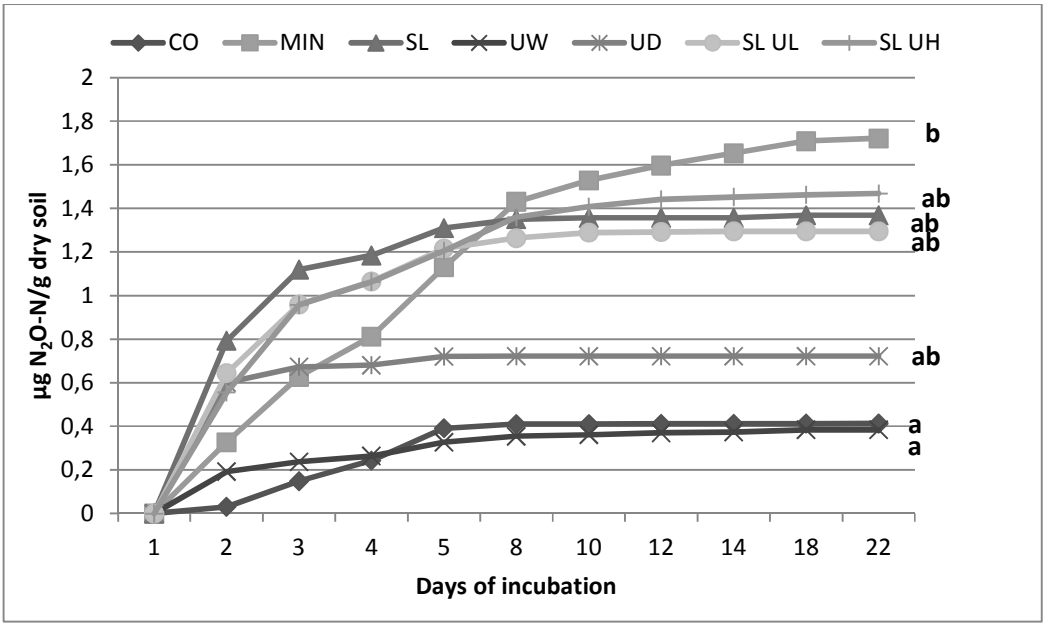

Figure 4: N_2O emissions during soil incubation. CO=non-amended control soil, MIN= soil + mineral fertilizer, UW = soil+wet *U. lactuca, UD*=Soil+dried and ground *U. lactuca*, SL= soil+biogas effluent from cattle manure, SL UL= Soil+biogas effluent from co-digested cattle manure with *U. lactuca* (20%); SL UH=Soil+biogas effluent from co-digested cattle manure with *U. lactuca* (40%).

N_2O emissions were clearly correlated to the mineral N-content of the different soil amendments, being highest in the minerally fertilized treatment and lowest in the non-amended control and pure *Ulva* treatments (Figure 4). No differences between the three types of digested slurries were detected. Due to erratic CO_2 measurements at the last sampling dates, CO_2 emissions are shown only for the first two weeks (Figure 5). They neither differed between treatments, except for the treatment that received the dried *Ulva lactuca* powder which caused a significantly higher CO_2 evolution compared to all other treatment except SL. The reason for that can only be speculated about, but might be a high content of easily degradable organic matter in the dried algae. The soils without organic amendment (CO and MIN) had correspondingly the lowest CO_2 emissions. Again, no significant differences between the different slurry types occurred.

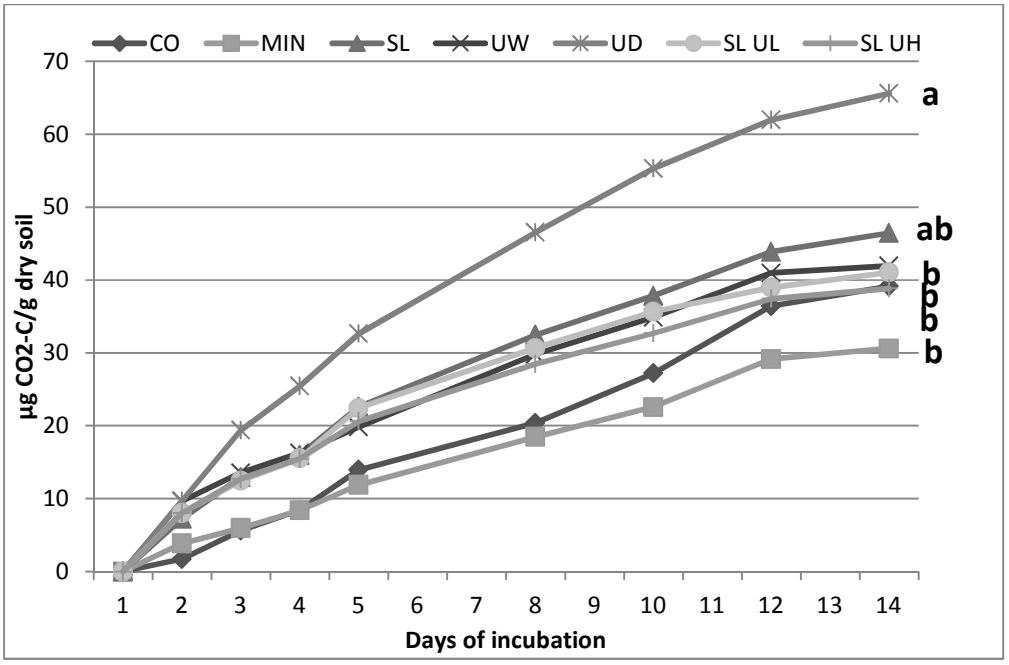

Figure 5: CO_2 emissions during soil incubation. CO=non-amended control soil, MIN= soil + mineral fertilizer, UW = soil+wet *U. lactuca, UD*=Soil+dried and ground *U. lactuca*, SL= soil+biogas effluent from cattle manure, SL UL= Soil+biogas effluent from co-digested cattle manure with *U. lactuca* (20%); SL UH=Soil+biogas effluent from co-digested cattle manure with *U. lactuca* (40%).

Preliminary Conclusions

The co-digestion of *U. lactuca* together with cattle manure did not alter the overall fertilization value and GHG emission potential of the digestate. However, some deeper insights in plant nutrient uptake and soil nutrient dynamics (including soil microbial biomass) are expected when all data are analysed.