Testing the effect of redirected glycerol by-products on the nutrition providing ability of the soil

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Abstract
During the production of biodiesel significant waste is produced of which the utilization is still to be solved. Such a material is glycerol. The experiment consisted of two periods. In the first period we tested the effect of the redirected glycerol by-product on the mineral nitrogen content of the soil in an incubation experiment of 2 weeks. In the second period for 2 months we tested the effect of the redirected glycerol by-products on the development of the plants with *Perennial Ryegrass* (Lolium perenne) indicator plant. Based on the tests it can be said that as an effect of adding glycerol, the active substance of the nitrogen fertilizer was immobilized. As the effect of 1% glycerol treatment the ammonium nitrate - the active substance of the nitrogen fertilizer - gets more than 50% immobilized in 2 days in the tested sandy soil. After 5 days, it gets technically 100% immobilized. The immobilized nitrogen is protected from being washed out, which is important for the nutrition, fertilizer technology and environment protection. The immobilization of the nitrogen can also be proved with the optical observation of the ryegrass and with the image analysis after the observation.

Key Words
Plant growing, pot experiment, image processing, soil biotesting.

Introduction

During the production of biodiesel a lot of waste is produced of which the utilization is still to be solved. Such a material is glycerol. The glycerol can be one of the important nutrients of the microorganisms in the soil. With that the nutrition of the soil can indirectly improve.
The carbohydrates and similar organic materials directed in the soil have a strong effect on the nutrition providing abilities of the soil. In particular this effect shows through the change of the amount of nitrogen that can be taken from the soil by changing the coal-nitrogen rate. If the C/N rate of the fresh material moves on a wide scale, the nitrogen gets temporary immobilized (Tisdale, Nelson 1966).
The nutrition providing ability of the soil can be tested with soil tests and plant experiments. The development rate of the plants reacts sensitively on the current nutrition supply. The development of plants, thus the dry matter accumulation, is not linear in time, in the vegetation period it is connected to certain stages of development (Lasztity et al. 1984, Waldren and Flowerday 1979, Prew et al. 1985), shows changes which are genetically determined yet influenced by external ecological factors, it is the result of the interaction af all these (Lasztity 2006). The growth rate depends significantly on the available nutrient (Jocic 1981, Lásztity and Kádár 1978).
The development of the plants can be followed with optical observation and computer processing. Narumalani rt al. (2009) and Auda et al. (2008) tried to gather information on the spread of the invasive plant species. Sanyal P and Patel (2008) judged the rice plant's health and feeding conditions by the shape and size of the plant. Timmermnas and Hulzebosch (1996) used image analysis for isolating the growth stages and plat parts. Behrens T and Diepenbrock (2006) scanned the development of the swede rape with the help of image analysis.

Methods

The test was made on a sandy soil from Fót. The main attributes of this soil: saturation percentage K_A=28.33, lime content ($CaCO_3$ %)=8 %, pH(H_2O)=8.2, humus content (H %)=1.4 %., AL-P_2O_5=95 ppm, AL-K_2O=120 ppm. For treatments we used analytical quality materials.
The experiment consisted of two periods. In the first period we tested the effect of the redirected glycerol by-product on the mineral nitrogen content of the soil in an incubation experiment of 2 weeks. In the second period for 2 months we tested the effect of the redirected glycerol by-products on the development of the plants with ryegrass indicator plant.
We used the following treatments:

- 0 or 100 ppm nitrogen treatment in the form of ammonium nitrate,

- 0 or 1% carbon treatment in the form of glycerol,
- constant 100 ppm phosphor (P_2O_5) and potassium (K_2O) treatment in the form of dihydrogen phosphate and potassium sulfate,
- constant distilled water according to 60% saturation percentage.

In all treatments the soil samples were mixed with solutions. The calculated amount of materials for the treatments was put in the soil, dissolved in this amount of water. We took samples from the soils of the incubation experiment 6 times, and dried them immediately to stop the processes. We took the samples on the 2nd, 5th, 7th, 9th and 14th day. To measure the mineral nitrogen content, we prepared soil extract by dissolving with 1 mol KCl solution. We measured the whole mineral (nitrate +ammonium) nitrogen content with a Parnas-Wagner steam distillator device, using $FeSO_4$ and $CuSO_4$ for NO_3 reduction. The effect of the treatments and the changes during the incubation time were detected by the evaluation of the calculated nitrogen contents with the two factor variance analysis. We tested the amount of nutrients that can be taken from the soil in pot experiments with ryegrass test plant. The test lasted for 8 weeks. Every week we optically evaluated the growth of the plants 3 times a week (Monday, Wednesday and Friday), we watered the pot according to their weight and randomly placed them in the greenhouse.

Results

Evaluation of the biotest on perennial ryegrass indicator plant

The images were made at 18 different times. On the plant images the image processing program counts the green pixels representing the color of leaves. In this way we could converse the plant growing status to numerical data. On the Image 1. there are the average values of the tested treatments as function of the days passed since the sowing, it is clearly visible that while the plants developed intensively during the nitrogen treatment (PKN), in absence of nitrogen (PK treatment) or after the immobilization of the nitrogen treatment by the glycerol, the development fell back significantly.

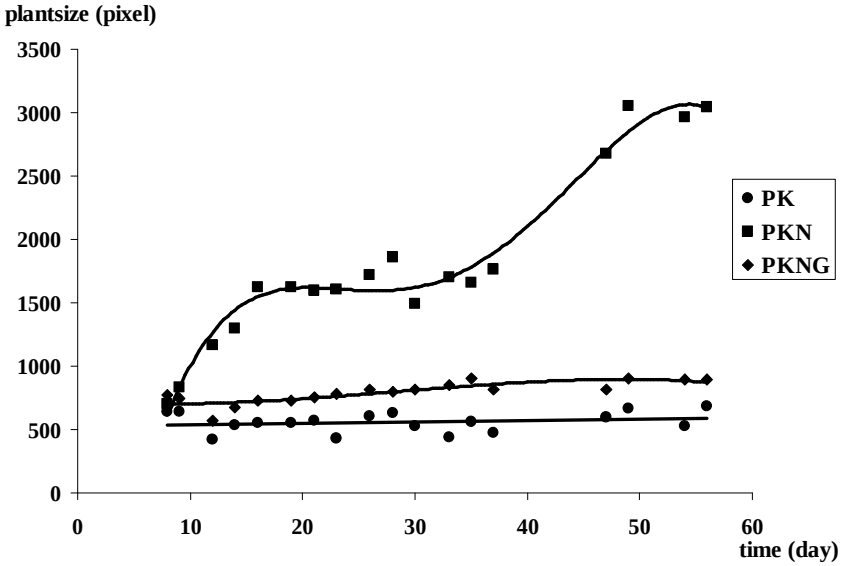

Image 1.: Development of perennial ryegrass as function of the days passed since the sowing in case of PK, PKN and PKNG treatments

Evaluation of the measured ammonium-nitrogen content

From the 36 samples of the incubation experiment and from the treatment solution we measured the nitrogen content. We evaluated the effect of the treatments and the incubation with analysis of variance. We tested the effect of the treatments with Fischer test and got the following results:

- The changes during incubation time – the effect of the factor A – is proved within 5% error probability (F-rate=4.0)
- The effect of the 3 nutrition treatments (PK, PKN, PKNG) – factor B – is proved within 1% error probability (F-rate=7.1)
- The interaction between the two factors (AxB) can be proved within 0,1% error probability. This latter shows that the changes during the incubation time depends significantly on the treatment given to the sample

For comparing the average values we used the SD(5%) values (table 1).

Table 1: Ammonium-nitrogen contents and their averages in mg N/kg soil

	2 days	5 days	7 days	9 days	12 days	14 days	B average
PK	3,2	7,8	7,4	5,3	5,3	4,9	5,6
PKN	26,2	12,2	9,8	4,1	2,9	2,5	9,6
PKNG	4,9	4,0	3,3	4,1	5,3	8,3	5,0
A average	11,4	8,0	6,8	4,5	4,5	5,3	

The ammonium nitrogen average values of the 3 nutrition treatments (factor B): for PK treatment 5.6 ppm, for PKN treatment 9.6 ppm, for PKNG treatment 5.0. The SD(5%) for this factor is 2.8, thus we can say, that the ammonium nitrogen content of the soil is not different after the PK and PKNG treatments. Compared to the PK and PKNG treatments the ammonium nitrogen content of the samples grew significantly after the PKN treatment. It is clear that that glycerol treatment (PKNG) neutralized the effect of the nitrogen treatment (PKN) as if the sample had not got any nitrogen treatment (PK).

Testing the effect of the time passed during the incubation process (factor A) (SD(5%)=4) we can see that while in the first week the ammonium nitrogen content of the soil samples decreased (11.4, 8.0, 6.8), it stagnated after the 7th day (6.8, 4.5, 4.5, 5.3). The conclusion is that the ammonium nitrogen content of treated soil already changes during the first week, after that it takes a little value. Testing the effect of the combination of the treatments we can see that in function of time the ammonium nitrogen with the PKNG treatment transformed before the first testing day (2nd day). The original ammonium nitrogen content of the nitrogen treatment (ammonium nitrate) is 50 mg. Thus the ammonium nitrogen contents of the PKN treatment did not change, just like in the case of the PK treatment (SD(5%)=6.9). In function of time the ammonium nitrogen content in the PKN treatment decreased significantly in the first 5 days keeps decreasing until the 9th day, than stagnates (SD(5%)=6.9). Summarized we can say that the conversion of the added ammonium nitrogen with nitrogen treatment significantly fastened as the effect of the glycerol treatment. The conversion happens in less than 2 days. Without the glycerol treatment the conversion takes a lot more time, more than a week.

Evaluation of the whole mineral nitrogen content (ammonium+nitrate)

From the 36 samples of the incubation experiment and from the treating solution we measured the whole mineral nitrogen content. We evaluated the effect of the treatments and the incubation with variance analysis. We tested the effect of the treatments with Fischer test and got the following results:

- The change as function of time – factor A – is within 10% error probability (F-rate=2.7), meaning it is rather like a tendency
- The effect of the 3 nutrient treatments (PK, PKN, PKNG) – factor B – is proved within 0.1% error probability (F-rate=1141.0)
- The interact between the 2 factors (AxB) can be proved within 0.1% error probability (F-rate=14.3). This shows that the change during the incubation time depends significantly on the treatment given to the sample.

We compared the average values with SD(5%) values (table 2.)

Table 2. Mineral (ammonium+nitrate)-nitrogen contents and their averages in mg N/kg soil

	2 days	5 days	7 days	9 days	12 days	14 days	B average
PK	15,6	19,0	22,0	23,1	22,7	24,2	21,1
PKN	86,7	115,7	101,9	108,0	98,6	123,5	105,7
PKNG	45,4	7,1	4,6	5,2	7,7	7,5	12,9
A averge	49,2	47,2	42,8	45,4	43,0	51,7	46,6

The whole mineral nitrogen average values as the effect of the 3 nutrient treatments (factor B): 21.1 ppm for PK treatment, 105.7 ppm for PKN treatment and 12.9 ppm for PKNG treatment. For this factor the SD(5%) is 4.5, thus we can say that the whole mineral nitrogen content of the soil samples is different in the case of the PK, the PKN and the PKNG treatments. After the PKN treatment the average of the whole mineral nitrogen content of the soil samples grew by the amount of the added nitrogen treatment (100 ppm). After the PKNG treatment the whole mineral nitrogen content of the soil samples decreased even compared to the results of the PK treatment. This shows that mixing the glycerol to the soil did not only immobilize the well dissolvable nitrogen content of the freshly given nitrogen treatment, but also part of the original whole nitrogen content of the soil.

Testing the effect of the incubation time (factor A) (SD(5%)=6.4) we can see that even though there are significant differences between the average whole nitrogen content of the soil samples but they show neither

decreasing nor increasing tendency as function of incubation time.
Testing the effect of the combinations of treatments we can see the without nitrogen treatment (PK) the whole mineral nitrogen content of the soil samples did not change after the incubation (SD(5%)=11.1). After the nitrogen treatment (PKN treatment) the whole mineral nitrogen content increased according to the treatment, by 100 ppm. Though as function of time there are significant differences between the whole mineral nitrogen contents, there is no tendency in it. We can see that the added well dissolvable nitrogen fertilizer did not get immobilized during the 2 weeks. Testing the effect of glycerol treatment (PKNG) as function of the incubation time we can see that after 2 days the added whole mineral nitrogen content (100 pp) got immobilized by more than 50% (there is 49.2% left). On the 5th day of incubation (7.1) all of the added nitrogen got immobilized along with the original (PK treatment) whole mineral nitrogen content of the soil.

Conclusion

Summarizing the evaluation we came to the following conclusions:

- The active substance of the nitrogen fertilizer gets immobilized after adding glycerol.
- On the tested sandy soil after adding 1% glycerol treatment, the active substance of the nitrogen fertilizer in form of ammonium nitrate gets immobilized in more then 50% after 2 days, and on the 5th day gets totally immobilized.
- The immobilized nitrogen is protected from being washed out which is important for the nutrition, fertilizer technology and environment protection.
- In 2 weeks the whole mineral nitrogen content of the nitrogen fertilizer in the form of ammonium nitrate did not change significantly on the tested sandy soil.
- On the tested sandy soil the mineral nitrogen content of the nitrogen fertilizer in the form of ammonium nitrate decreases significantly for 5 days, until the 9th day it shows decreasing tendency then stagnates on the balanced values of the untreated soil.
- The immobilization of the nitrogen can be proved by the optical observation of the growth of the perennial ryegrass and after that by the image analysis.

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