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RESEARCH ARTICLE

Algae production on pig sludge

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Abstract This report shows that pig manure can be used for algal production in small farms. Huge pig sludge amounts produced using actual methods in large-scale pig breeding farms is a major issue due to the lack of disposal options and potential water pollution. This issue may be solved by using pig sludge for algal biofuel production. Therefore, we studied an economical method of algae production on pig sludge that can be operated on animal farms in Hungary with modest levels of investment. We analyzed four algae species, Chlorella vulgaris, Scenedesmus quadricauda, Scenedesmus dimorphus, and Arthrospira platensis, in the laboratory and C. vulgaris in outdoor conditions. The following parameters were studied: pot size, illumination, temperature, filtered versus unfiltered pig manure, water depth, aeration, CO₂ enrichment, inoculums solution, fertilization level, and length of rotation period. Produced alga biomass was measured every 4 days, and the protein- and lipid content was analyzed using the method of Bradford (1976) and Erickson (1993). Our results show first that the unfiltered pig manure is not suitable for algae production due to illumination shortage. By contrast, using filtered pig manure, we found that C. vulgaris yield in 70-1 indoor pots was 64% higher than in smaller pots. In a larger outdoor system, the annual dry yield of C. vulgaris reached 141-259 t/ha during 12-day-long rotation periods. Here, we demonstrate that only filtered liquid pig manure can be recommended for algae production. C. vulgaris is the most suitable alga species for use in large-scale experiments on pig sludge. Based

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University of Debrecen, e-mail: abai@agr.unideb.hu on our outdoor experiments, the adaptation of a 12- to 14day rotation period could be considered the most reasonable. An algae farm can therefore be operated with relative modest amount of capital, can hence address the issue of sludge management, and provide a substrate for energy production.

Keywords Algae · Open pond · Sludge management · Chlorella vulgaris

1 Introduction

The consequence of commonly used technologies in largescale pig breeding is a significant amount of sludge, the inefficient disposal of which strongly threatens the environment. Liquid manure is a by-product produced in large quantities with high organic material content. Its composition and nutrition content are highly variable, depending on pig species and farming technology used. Most of the manure is composed of feces, urine, drinking and processed water, as well as various other waste materials. The most common way to use liquid manure is to spread it on farm lands, in order to enrich the soil and compensate for the nutrient and water requirements previously grown crops took from the soil. However, there are several other methods of treating liquid manure, such as making compost, biogas production, aerobic digestion, or filtration; however, the costs of these procedures are extremely high (Sevrin-Reyssac 1998). This considerable level of costs is the reason why algae-based sludge management has been studied since the 1960s (Golueke and Oswald 1962; Lau et al. 1995; Tam and Wong 2000); in spite of this, the use of the various kinds of waste water and agricultural by-products in algae production has only recently become significant (Wilkie and Mulbry 2002; Mulbry et al. 2008; Kim et al. 2010). This is



mainly because of the growing role of algae in biofuel production and the recognition that using certain algae species in feeding animals has great advantages. Typical content of fatty acids of differential *Chlorella* species is between 15% and 25% (Petkov and Garcia 2007); in the case of other microalgae species, it is between 14.8% and 38.9% (Gressler et al. 2010). Production of those microalgae species which are rich in lipids can contribute to the reduction of global warming thanks to the capture of great amount of CO₂ and their utilization as nextgeneration biofuel (Ördög 2009).

According to Barlow et al. (1975), the composition of liquid pig manure is favorable to algae production. Among algae species, it is the *Chlorella vulgaris* (Beijerinck) which can produce the greatest amount of biomass on this kind of nutrient substratum. Pig manure is extremely rich in ammonium nitrogen, the form of nitrogen which this algae species can most efficiently utilize (Capblancq 1982).

The aim of algae experiments, which have been carried out by the University of Debrecen in cooperation with the Monergo Ltd since 2009, was to devise an economical form of alga production technology which can be profitably operated on pig farms in Hungary with modest levels of investment. However, the idea is not new; several previous experiments are known regarding algae production from pig sludge (Barlow et al. 1975; Fallowfield and Garrett 1985; De Godos et al. 2009), but the approach may be regarded new, the realization of it on cost-effective way at industrial level could be considered as a new research area, especially in shortage of capital and in Hungarian conditions. To this end, we carried out laboratory and outdoor experiments using four algae species (C. vulgaris (Beijerinck), Scenedesmus quadricauda (Turpin), Scenedesmus dimorphus (Turpin), Arthrospira platensis (Gomont)) and accompanying, complex economic assessments on the basis of our results (Bai et al. 2010).

2 Materials and methods

2.1 Our laboratory experiments (2009–2010)

All laboratory experiments—except the last one—took place in 14-day periods, with the following parameters:

- 1. Test year is 2009; the purpose is to produce enough algae vaccine under generally recommended conditions
 - 1.5-1 pot with 1.3 1 of water
 - 77 to 126 mg wet weight/100 ml of inoculum solution
 - 10 ml nutrient solution (detailed, NO₃–N 39.60 g/l; NH₄–N 44.40 g/l; water-soluble phosphorus pentoxide 36.00 g/l; water-soluble potassium oxide

- 72.00 g/l; B 0.12 g/l; Cu 0.24 g/l; FeEDTA 0.60 g/l; MoEDTA 0.12 g/l; ZnEDTA 0.24 g/l)
- Illumination, 4,500 lx, 18 h/day
- Temperature, 24°C
- Aeration, 50 l/h
- 2. Test year is 2009; the purpose is to study the effects of changes of illumination.

The following parameters are the same as for the previously mentioned test:

- 77 to 126 mg wet weight/100 ml of inoculum solution
- Illumination, 4,500 lx, 18 h/day
- Temperature, 24°C
- · Indoor conditions

Different parameters, compared with the previous test:

- Unfiltered liquid pig manure: 12.5% organic matter, 4,880 mg/l nitrogen, 2,968 mg/l, phosphorus, 2,969 mg/l potassium, which is much thicker than the typical animal sludge (300 mg/l nitrogen, 100 mg/l phosphorus, 260 mg/l potassium, (Nábrádi 2002))
- 5-1 pot with 20 cm diameter
- 180-l aquarium with 3 cm water depth
- Aeration, 100 l/h
- 3. Test year is 2009; the purpose is to analyze the effect of dilution of manure and carbon dioxide fertilization.

The following parameters are the same as for the previous test:

- 77 to 126 mg wet weight/100 ml of inoculum solution
- Illumination, 4,500 lx, 18 h/day
- Temperature, 24°C
- Aeration, 100 l/h
- 5-l pot with 20 cm diameter

Different parameters compared with the previous test:

- Diluted liquid manure: in the form of 25%, 10%, 5%, and 1% solution
- Carbon dioxide enrichment: ad libitum and zero dosing at all settings
- Manure solution diluted in four ways, two repetitions in each case
- Test year is 2010; the purpose is to choose the alga species which can replicate themselves best at the larger test scale.

The following parameters are the same as for the previous test:

77 to 126 mg wet weight/100 ml of inoculum solution





- Illumination, 4,500 lx, 18 h/day
- Temperature, 24°C

Different parameters compared with the previous est:

- 12-day measurement interval (June 28–July 10, 2010)
- 70-1 tanks, 20 cm water depth
- No pig manure and carbon dioxide dosing, 500 ml of medium (contains 0.84 mg/l nitrogen, 0.36 mg/l phosphorus, and 0.804 mg/l potassium as semi-intensive technological version) was replaced
- Moderate aeration, 50 l/h

The effect of differential light intensities and pH was not studied during the experiences; we tried to assure the optimum level based on the results of earlier sources. According to Ghezelbash et al. (2008), the maximum yield can be expected at 4,500 lx of light intensity, and Seyfabadi et al. (2010) adjust illumination also on a similar scale (100 photons/m²/s \sim 5,000 lx) in their examinations). The importance of illumination of pig sludge and the differences between laboratory and outdoor pilot-plant culture are discussed in the study of Fallowfield and Garrett (1985), with particular reference to retention time, algal productivity, and the level of NPK removal.

The protein content of algae biomass was analyzed using the method of Bradford (1976), while the lipid content of algae biomass was determined by the method of Erickson (1993) detailed by the referred sources.

2.2 Small-scale outdoor experiment (2010)

Based on the results of the previous tests, our experiments with *C. vulgaris* were also performed at a larger scale and under outdoor circumstances. The use of this species is also recommended by several related sources (Barlow et al. 1975; Fallowfield and Garrett 1985; De la Noüe and Bassères 1989). The objective was to achieve realistic yield figures, which can be expected to occur when applying large-scale technologies. By using these figures, the expected economic indicators can be estimated with reliable accuracy. During the experiments, we also examined how the aeration and nutrient supply affected the yield since these expenses can significantly influence the economics of production.

The experimental parameters are all different from the circumstances of the previous laboratory experiments:

- Larger dose of vaccine than for the previous tests, 3.84 g wet weight/500 ml (*C. vulgaris*)
- 28-day measurement interval (from July 9 to August 6, 2010)
- A repetition for each variable (three levels of nutrients, two types of aeration, a total of 12 tanks)

- 1,000-1 tanks, 500 1 of water, 30 cm water depth
- Natural illumination (14–15 h/day)
- Outdoor temperature (17–29°C daily average temperature, min.14°C, max.35°C; 28-day average, 24.5°C)
- No added manure and carbon dioxide; instead, we used 250, 500, and 1,000 ml nutrient solutions with 0.42 mg/l nitrogen, 0.18 mg/l phosphorus, and 0.402 mg/l potassium on the basic case (250 ml)
- Moderate aeration, 50 l/h and without aeration in each of the nutrient level.

The ordered filtration equipment did not arrived in time; that was the reason why the tests of 2010 were performed with manure-free nutrient solutions with similar illumination characteristics to filtered manure.

During the experiment, the external temperature was measured every 4 h. Based on the data, we determined the minimum, maximum, and the average daily temperatures every day. The dry weight of produced alga biomass was also measured every 4 days, based on the samples taken from each basin after filtration and evaporation of water.

The results of the CO_2 treatment and aeration were evaluated using the SPSS program. The effect of CO_2 treatment, nutrient supply, and aeration was calculated by analysis of variance (one-way ANOVA). To determine the optimal growing interval, Pearson's correlation test was performed.

3 Results and discussion

Experiment no.1 provided sufficient alga quantity for the later experiments, but the level of the inputs was not evaluated as being economical, so the construction of large-scale technology cannot be recommended here. Based on the results of experiment no. 2, we found that in producing algae on liquid pig manure, the nutrient concentration of undiluted liquid manure is not the main limiting factor, but light, thanks to its impact on photosynthetic activity. Consequently, completely liquid pig manure is not suitable at all for algae production at a large scale, only diluted or filtered liquid pig mature can be used.

The results of experiment no. 3 have shown that higher nutrient concentrations can result in higher yields, but the protein content of the biomass increased at the same time, leading to the reduction of lipid content in the case of the oil-producing alga species (*S. dimorphus*). This result is in accordance with the experimental results of Ördög et al. (2011). It should be noted that the protein content of a marine microalga culture (*Tetraselmis suecica* (Kylin)) significantly grew when the salinity increased





for a given nutrient concentration up to 20‰, but from this point the process was reversed (Fabregas et al. 1984).

Furthermore, when adding pure CO₂, the quantity and protein content of biomass increased as opposed to changing of the lipid content (Fig. 1). Regarding previous experiments about the cultivation of *A. platensis* using CO₂, (Soletto et al. 2008), a too large amount of CO₂ caused biomass growth to be inhibited by excess carbon level, likely due to salinity and osmotic pressure increase.

While the impact of the sludge concentration was not confirmed by the statistical tests because of the small number of samples (parallel tests were not carried out), this does not mean that the concentration of the liquid manure solution cannot affect the composition of each species.

The results of statistical analysis (one-way ANOVA) of CO_2 fertilization clearly showed that the CO_2 -treated samples produced a significant yield surplus in cases of C. vulgaris, S. quadricauda, and S. dimorphus (level of significance was 0.009, 0.012, 0.033, respectively, and F value exceeded the critical 5.59 level in all cases). In case of A. platensis, the level of significance was 0.057, so CO_2 treatment should not be considered significant.

To determine the economics of the added carbon dioxide gas, it is recommended to compare the costs of the utilized gas, the amortization of the investment required for application, and also the cost of electricity with the financial value of the additional yield (compared to the control figures). The significant part of the cost (80% to 85%) is that of carbon dioxide. The separation of the costs should be reasonable because if we add our own produced gas (created as a by-product), we have only the cost of the application. On the other hand, if we used purchased gas, all of the costs will appear. In the case of large-scale application, these figures should be larger than the data of the experiments, due to the following reasons:

- If we use waste carbon dioxide, the investment costs (its part per year), and especially the costs of the pipeline construction, would be much higher This would be particularly true in cases in which we could not put our own gas into the system, but we use the gas received from a neighborhood supplier.
- If we use purchased gas, we might not be able to determine the actual carbon dioxide demand, and removing any surplus—this being a dominant cost element—could cause a very substantial increase in costs.

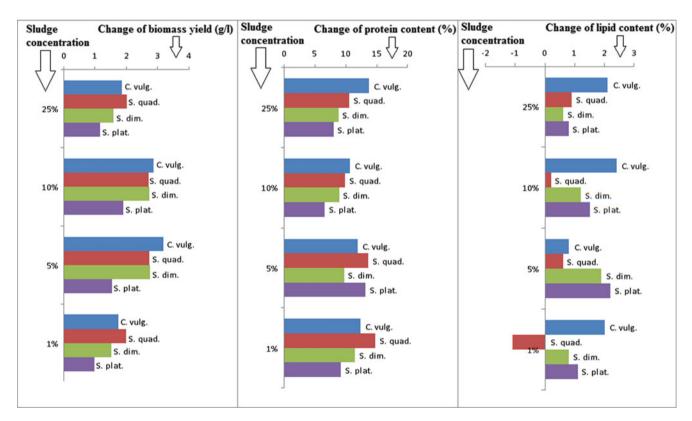


Fig. 1 Effects of CO2 treatment and sludge concentration on the biomass yields, protein and lipid content of the examined species. The effect of CO2 treatment was calculated by subtraction of the yields

of control samples from the yields of treated samples. Ten percent sludge concentration and *A. platensis* resulted the maximum yield





The economy in the case of purchased gas proved to be unreasonable for each examined species and in most cases caused losses. The income coming from using waste gas in optimal cases—depending on the species—can reach up to EUR 0.073–0.124 per day per m³, thus in the case of a farm using 10% concentration of manure and handling, 70 m³/day liquid manure, the yearly profit, in principle, could reach 25,500–29,100 EUR profit compared with the untreated versions. The extra costs of the experimental investment and of the transfer of flue gas from a close power plant can be expected to return within a year or two.

Having the previous experimental data in experiment no. 4, we wished to determine whether the alga species used in the previous tests are capable of making the earlier levels of yield in larger quantities. We thus performed tests on manure-free nutrient solutions supplied with the applicable nutrient elements. The tests of 2009 and 2010 have proved that use of diluted manure results lower yields than filtered sludge. The daily average yield results are described in Table 1.

In case of three species, the results did not differ significantly from the average of the previous experiments; however, *C. vulgaris* surpassed it by 64%. This result is mainly owing to the high level of adaptability of this species. The other species gave much worse results, not only in terms of their yields but in contamination levels, as well.

The data obtained made it clear that it is the *C. vulgaris* which should be primarily applied in large-scale experiments if our goal is to produce larger and more consistent amounts of biomass. Therefore, open-field experiments were carried out with this alga species. However, it should be noted that if we are to produce especially algoil, the use of the *Scenedesmus* species also should be considered. In spite of their smaller biomass weight, this species has such a great amount of lipid content that it can exceed the lipid yield of the *Chlorella* species (Table 1, last column). When producing the *Scenedesmus* species, the main task should be to avoid contamination by other species because *Scenedesmus* species are less dominant. Any final result would be a mixed and less valuable culture, instead of a *Scenedesmus* culture.

Table 1 The change of the daily average dry yields of labor experiments (grams per cubic meter). In case of 2010 laboratory experiments in 70-l indoor pots with filtered pig sludge, *C. vulgaris* surpassed the

Based on the results of the outdoor experiments, the following general conclusions can be made:

- Nutrition supply has a significant yield-increasing effect up to the optimum point, while the aeration level brings only moderate and uncertain growth.
- Temperature changes during the measurement period (17–29°C) practically did not affect the reproduction of the algae, so no yield reduction can be expected during the summer months.
- Alga yield increases significantly until the12th day, thereafter—especially in the case of the variants receiving moderate or small nutrient supply—we expect the decay of algae or significant reduction in yields.
- We have proved statistically that the production period between the 12th and 16th days should be taken the most favorable, as has been supported by a correlation analysis. We obtained the strongest correlation (r=0.912and 0.877, where the Pearson's correlation coefficient was 0.000 in two repeated returns) during the 12-day period, while the correlation coefficients were slightly stronger (r=0.848 and r=0.819) in significance under 0.000 during the 16-day-long growing season. In the case of the 20-day-long period, there was a very significant decrease in the value of the correlation coefficient. The reason for this decrease is the risk of the algae system's collapse. The possibility of such an event increases simultaneously over time as the algae system grows. Faster rotation does not only reduce the likelihood of this occurrence but it also allows the handling of a greater quantity of liquid manure by the algae.
- The results of the repeated tests compared to the control results strongly vary, in spite of the practical equality of the test conditions.
- Larger systems have better adaptability and a capability to neutralize temperature fluctuations and other harmful effects. As a result, the annual average yield in all but one case of our measurements surpassed not only the best results of the liquid manure tests (125 t/ha) but also the results of the previous nutrient solution laboratory

yield of the previous experiments (in smaller pots with diluted manure) by 64%; however, the results of the other species did not differ significantly from the previous ones

Species	2009 diluted manure solution				2009 average	2010 NPK solution	Difference %	2010 lipid
	25%	10%	5%	1%				
C. vulgaris	134	209	164	127	158	260	64	47
S. quadricauda	140	236	209	134	180	164	-9	48
S. dimorphus	116	206	159	89	143	153	8	51
A. platensis	92	167	142	74	119	101	-15	15





experiments (156 t/ha), at the same (30 cm) water depth and 200-day vegetation period. Most of the results are approximately at the same level as the average yield obtained by applying CO₂ in a liquid manure solution (248 t/ha); in three cases (1, 5, 6i), the result is even significantly higher (Table 2).

Since the 20-day rotation period brings complete decay of the algae near half of the variants, we examined only the economic indicators of the growth period ending on July 25. After this date, the risk of alga production would sharply increase and the average yield would also decrease except one variant of an alga produced with the most intensive nutrient supply. The possibility of the 20-day-long rotation period should be examined only in this variant, although the expected 11% additional yield (compared to the 16-day period) would probably not be worth the much higher production risk. In the case of rotation periods shorter than 12 days, the decrease in yields (and in neutralization of sludge) is significant; thus, the application of such rotation periods cannot be recommended. If we compare the rotation periods that can be taken into account in practice, we can state the following:

• In the case of the 12-day-long period, the application of a medium-level nutrition supply with no aeration can be recommended. This will bring a higher yield in alga

- production until the 14th or 15th days, compared to the variants with much higher expenditures (aeration, more intensive fertilization).
- After the 15th or 16th days, the most intensive (1+) technology brings the maximum yield.

According to our experimental data, the positive effects of aeration can be experienced especially during the initial period, with a smaller quantity of algae. After 12 days, this effect is completely gone, and the aeration in case of intensive fertilization even reduced the quantity of algae. To choose the optimum rotation period, we need to know the cumulative values of the multiplication compared to the basic figures since these values will show us which variant and how many days will give the maximum yield and revenues (Fig. 2).

According to the one-way ANOVA analysis, there is no significant difference in terms of the yields of the aerated and non-aerated ponds. Level of significance considerably exceeded the critical value (0.05) in both repetition (0.370 and 0.142); consequently, the effect of aeration statistically cannot be proved. The reason for this may be the small size of the ponds because the professional and efficient operation of large-scale ponds definitely requires adequate ventilation (mixing/convection).

To determine whether the nutrient solution concentration influences the yields of the variants, the variant analysis (ANOVA) did not show statistically reliable

Table 2 The annual dry yields of open field experiments. In outdoor system, the annual dry yield of *C. vulgaris* reached 141–259 t/ha during 12-day-long rotation periods, depending on aeration and fertilization level

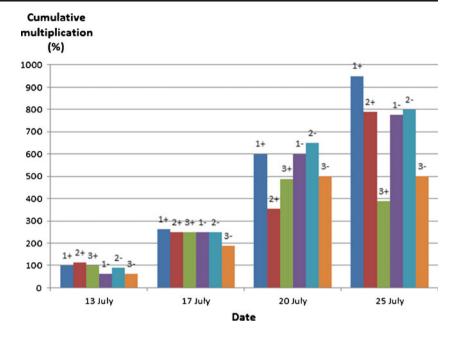
Number of		Biomass (t/ha/200 day, 30-inch water depth)						
treatment	July 9	July 13	July 17	July 21	July 25	July 29	August 2	August 6
1.	5	115	144	250	<u>281</u>	<u>311</u>	211	86
1. i	5	115	158	211	<u>266</u>	0	0	156
2.	5	144	158	230	<u>252</u>	242	288	66
2. i	5	115	130	141	<u>202</u>	184	115	0
3.	5	86	115	173	<u>223</u>	0	0	0
3. i	5	144	173	<u>202</u>	0	0	0	0
4.	5	58	144	240	<u>252</u>	236	192	132
4. i	5	86	144	<u>221</u>	194	207	202	160
5.	5	86	158	269	<u>274</u>	72	197	0
5. i	5	115	130	<u>230</u>	187	0	0	0
6.	5	58	86	<u>125</u>	0	0	0	0
6. i	5	86	130	259	<u>288</u>	190	182	30

In case of longer rotational period, the risk of alga production significantly increased. Underlined values, the maximum value of rotation i repeat, 0 algal mortality; (1), (4), 1 1/0.5 m³; (2), (5); 0.5 $1/m^3$; (3), (6), 0.25 1/0.5 m³ nutrient solution, white cells aeration basins, gray cells non-aerated ponds





Fig. 2 The extent of cumulative multiplication of the alga yields compared to those of the control group (yield of July 9). The efficient operation of large-scale ponds definitely requires adequate ventilation and extensive or semi-intensive fertilization level



differences (0.398 and 0.871). The small size of the ponds used in the experiment may explain probably the lack of satisfactory results, so we can assume that utilization of a 0.25-l nutrition solution would have been sufficient and the application of lower concentration would have shown significant differences.

Examining the economic results of the 16-day-long vegetation period, the economic factors in Table 3 should be compared between the first variant with aeration (1+) and the second variant without aeration (2-). The data cover a 1-year-long period (a 200-day growing season), a 1-acre size alga pond, and 30 cm of water depth.

In sum, based on our field experiments, the 12- to 14-day rotation periods with middle-level nutrition doses, without

Table 3 Comparison of economic indicators for a 12-day-long and a 16-day-long rotation period

Surplus input	Unit	2-/1+ variant
Additional revenue ^a Aeration cost ^b	EUR/ha/year	14,935 (-) 9,382
Additional cost of fertilizer ^c		(-) 3,713
Total	3	1,840
Reduction of the sludge processing capacity ^d	m³/ha/year	(-) 43,636

Comparison of the two most promising technology (2-, 1+) shows that use of the first one (with middle level of fertilization and aeration in longer rotational period) could be recommended

aeration (2- variant), using of the liquid pig manure as a nutrient and slow mixing instead of aeration would be the most reasonable possibilities.

4 Conclusions

Based on the results of our laboratory experiments, we found that whole liquid pig manure is not suitable at all for large-scale algae production; only diluted or filtered liquid pig mature can be used; we recommended the last one. Our results have shown that higher nutrient concentrations and additional CO₂ can result to higher yields, but the protein content of the biomass increased at the same time and led to the reduction of lipid content in the case of the oil-producing alga species (*S. dimorphus*). The data obtained show that it is the *C. vulgaris* which should be primarily applied in large-scale experiments if our goal is to produce larger and more consistent amounts of biomass at moderate risk.

Based on our outdoor experiments (*C. vulgaris*), nutrition supply has a significant positive effect on algae yield up to the optimum point, while the aeration level brings only moderate and uncertain growth. Twelve- to 14-day-long rotation periods would be the most reasonable possibilities with middle-level nutrition doses, using liquid pig manure as a nutrient, which probably results in larger yields and profits than the more intensive technological variants (with aeration and more fertilizer).

Aeration is not recommended; instead, slow mixing is recommended. The high-level nutrient supply might explain a slightly longer rotation period.





^a 311 t/ha/year, 274 t/ha/year, 403,64 EUR/t price

^b 1.56 €cent/m³ /day, 200 days per year, 3,000 m³ /ha

 $^{^{\}rm c}$ 840 g N, 360 g P, 804 g K per rotation, 1+:13 rotation/year, 2–:17 rotation/year

^d 3,000 m³/ha, 1+:13 rotation/year, 2-:17 rotation/year

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