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Effect of Composting on Substrate Quality, Mycelial Growth and Mushroom Yield: Case of *Pleurotus Ostreatus Var 2175* Produced Respectively on Broken Cottonseed and Composted Grevillea Sawdust

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Abstract

This research aims to improve the productivity of oyster mushrooms by improving the quality of the culture substrate given that contamination continues to be noticed despite the fact that pasteurization has been well conducted. The study took place in the food microbiology laboratory of the Faculty of Agronomy and Bioengineering and in the organic chemistry laboratory of the Faculty of Sciences at the University of Burundi. The main objective of our study was to highlight the effect of composting on the quality of the substrate, on mycelial growth and on the yield of fungi in the case of *Pleurotus Ostreatus Var 2175* produced respectively on broken cotton seeds and composted Grevillea sawdust.

The hypotheses posed were to see if the composting of the substrate makes it possible to create a selective medium which promotes the mycelial growth of the oyster mushrooms to the detriment of bacteria and competing and antagonistic moulds.

Before the experiment, the substrate contained Bacteria, *Rhizopus, Tricoderma, Aspergillus Flavus and Aspergillus Niger*, but during composting we observed an elimination of these microorganisms.

The results obtained proved that composting makes it possible to create a selective medium which favors the mycelial growth of oyster mushrooms to the detriment of competing and antagonistic bacteria and moulds.

Nevertheless, it is preferable to supplement the substrate thus treated with nitrogen-rich supplements to readjust its C/N ratio since the latter deviates considerably from the value required for oyster mushrooms during composting.

Keywords: Substrat, Myciculture, Compostage, Pleurotes, Brisures, Moisissures

Introduction

Estimated at around 12 million in 2019, the Burundian population continues to grow. And according to ISTEEBU projections, it will have doubled in 2050. This is normal for a country with a growth rate of 2.4% and a fertility rate of 5.5 children per woman according to this same institution. This situation has serious consequences. At the top of the list, poverty in full swing. Indeed, with a poverty rate estimated at 74.7%, Burundi is among the 5 poorest countries on the planet and has a malnutrition rate of 60%. Here, it is without mentioning the unemployment figures, that other time bomb, due in part to this uncontrolled galloping demography (NIMPAGARITSE P, 2019) [1].

Faced with this galloping population growth leading to a considerable reduction in cultivable land, the exaggerated overexploitation of land, the growing reduction in production and soil fertility, it is necessary for the population to start producing crops. with high nutritional added value and short crop cycle such as mushrooms and vegetables (Shrijan *et al*, 2013).

Thus, mushroom cultivation has been identified as a potential sector offering various benefits to poor farmers in settings where low land availability is a production constraint. They can not only be grown for nutrition and food security, but also to improve livelihoods through income diversification and job creation (Gateri *et al*, 2009) [2].

This research is part of the improvement of the productivity of oyster mushrooms by improving the quality of the culture substrate given that the contaminations continue to be noticed despite the pasteurization being well carried out. A cultivar *Pleurotus ostreatus var 2175* was used for this study.

With this in mind, a composting of the substrate was carried out for 14 days to test the influence of the high temperatures that occur during the fermentation process on the elimination of microorganisms from the substrate.

The results obtained showed a clear reduction in molds in the substrate during composting as well as a large proportion of bacteria.

However, the same results showed that the composting process leads to a considerable reduction in the nitrogen rate in the substrate, which increases the C/N ratio, which moves away from the value of 50 required for oyster mushrooms. It will then be necessary to supplement our substrate with nitrogen-rich supplements such as rice or wheat bran and chicken droppings for example.

Material and Methods

The experiments were carried out at the Faculty of Agronomy and Bioengineering of the University of Burundi; in the food microbiology laboratory, in the premises of the former faculty of agronomic sciences (FACAGRO) and in the organic chemistry laboratory of the Faculty of Sciences.

This area enjoys a relatively hot and humid climate as well as an altitude varying between 774m and 1000m. The study focused on a single cultivar of *Pleurotus ostreatus* (cultivar 2175) on broken cotton seeds and composted Grevillea sawdust.

It should be noted that Pleurotus ostreatus is one of the most cultivated species of mushrooms in soilless conditions throughout the world (KIYUKU *et al*. 2013) [3].

The analyzes consisted in carrying out, on the one hand, the composting of the substrate and monitoring the variation in temperature during this composting and, on the other hand, in determining the effects of the variation in temperature during the composting time. on the microbial population of the substrate and on its nitrogen and total organic carbon content.

We hypothesized that composting the substrate creates a selective environment favorable to mycelial growth of oyster mushrooms to the detriment of competing and antagonistic bacteria and molds.

We chose to do the study on Pleurotus ostreatus because it is the most cultivated species currently in Burundi. The strains were given to us by the promoter of the subject. The substrates used and especially broken cotton seeds were preferred because they are the most productive among the local substrates.

Biological Material

The biological material used was composed of a single strain of oyster mushrooms: Pleurotus ostreatus 2175. The biological material used was composed of two types of substrate, namely: broken cotton seeds and Grevillea sawdust.

Laboratory Equipment

The laboratory equipment we used was grouped into three categories according to activity:

- The material used for composting consisted of:
  drum for soaking the substrate; a tarpaulin for the constitution of the heap for the fermentation; a food thermometer to take the temperature at various time intervals.
- The laboratory equipment used for the microbiological analyzes consisted of:
  A well-ventilated laminar flow hood; A precision 0.01g scout-pro electronic scale for measuring reagents; A magnetic stirrer for the homogenization of solutions; An autoclave for the sterilization of culture media; Erlenmeyer flasks for autoclaving culture media; Test tubes to perform different decimal dilutions; Vortex for homogenization during dilutions; Micropipettes; A STOMACHER device for extracting juice from substrates; A colony counter; Petri dishes; An incubator; Parafilm paper.
- The laboratory equipment used for the physico-chemical analyzes consisted of:
  Mortar and pestle; 800 µm mesh sieve; Steamroom; Cruet; Burette; Stand; 0.01g precision electronic scale; 500ml Kjeldahl flasks; Analytical balance at 1/10th mg; Kjeldahl attack ramp; Laminar flow hood; Calibrated 250,1000 and 2000 ml; Funnels; Pipette 25ml; Steam distillation apparatus(es) (50ml cell, B19 running-in) Parnass type; 100ml Erlenmeyer flask; Burette for titrating methröem 0-5ml (division 0.005ml); Stirrer and magnetic sticks.

Reagents Used

- The reagents used for the carbon assay consisted of:
  Potassium dichromate: K2Cr2O7; Ferrous Sulfate: FeSO4.7H2O; Concentrated sulfuric acid (95%): H2SO4; Concentrated orthophosphoric acid (85%): H3PO4; O-Phenanthroline monohydrate: C12H8N2.H2O; Ferroin indicator; Naphthalene.
- The reagents used for the carbon assay consisted of:
  Concentrated sulfuric acid cc p.a. (95%): H2SO4; Copper sulphate p.a. (CuSO4.5H2O p.a.); Potassium sulphate p.a. (K2SO4 p.a.); Iron sulphate p.a (FeSO4.7H2O p.a); Selenium powder p.a; Caustic soda flakes p.a (NaOH p.a); Phenolphthalein; Titrisol H2SO4 0.1N; Sodium carbonate p.a (Na2CO3 p.a); Bromocresol green p.a; Methyl red p.a; Boric acid p.a (H3BO3 p.a); Parafilm.

  The role of this soaking is to soak the substrate to allow good invasion of the mycelium. It also aims to eliminate, by leaching, easily assimilated sugars likely to attract microorganisms (bacteria, yeasts and molds) that prevent the proper development of the mycelium (Kiyuku *et al*. 2020) [4].

  After draining, we dumped the substrate on a tarpaulin and proceeded to the spin test and noted the initial temperature.

  Coming out of the bag, the substrate was too compact and we tried to mix it to make it homogeneous in order to promote its aeration.

  Finally, we formed a pile not exceeding 1 meter in height and we set ourselves the following turning program: first turning after 5 days then follow the turning each time after 3 days until the appearance of actinomycetes which is accompanied by the significant decrease in temperature and a change in smell; signs marking the end of composting. We stopped at the 14th day of composting.

Microbiological Analyzes

The microbiological analyzes were carried out in the food microbiology laboratory of the Faculty of Agronomy and Bioengineering at the University of Burundi. They focused on determining the microorganisms present in the substrate before composting and monitoring their qualitative and quantitative evolution during composting.

- The analysis samples were obtained by mixing, using

the SOMACHER at 230 revolutions per 2min, 5g of wet substrate with 45ml of peptone water;
- For the evaluation of the bacterial flora in our substrate, we cultivated them on the PCA culture medium then incubated at 37°C for 24 hours.
- For the evaluation of the competing fungi in our substrate, we cultivated them on the PDA culture medium then incubated at 25°C for 72 hours.
- Enumeration was performed using a colony counter.

Physico-Chemical Analyzes

The physico-chemical analyzes were carried out in the organic chemistry laboratory at the Faculty of Sciences at the University of Burundi. They focused on the measurement of the evolution of the nitrogen content of the substrate during composting, the measurement of the evolution of the carbon content of the substrate during composting and on the evolution of the C/N ratio.

- The determination of total nitrogen was carried out by acid mineralization of the dry substrate by the Kjeldhal method according to the AFNOR ISO 11261 standard on samples dried at 105°C for 24 hours then finely ground and sieved at 800 µm mesh;
- The determination of carbon was carried out by the oxidation method or Walkley-Black method;
- The C/N ratio was determined by relating the values obtained for carbon to the values obtained for nitrogen.

Statistical Analysis of Results

The statistical processing of the results (data) was performed using SPSS version 20.0 software and focused on certain tests and analyzes that are compatible with a low level (less than 50 units) of statistical units (number of observations done). We needed to compare averages of evolution (decrease) of microorganisms (SM), correlations between temperature and evolution of bacteria, temperature and evolution of molds, temperature and evolution of carbon and nitrogen.

Thus, for example, for the comparison of the means, the analysis of variance (1-factor ANOVA) with respect to the three batches of substrates, demanding with regard to the size of the sample (equal to or greater than 20 units/observations), could not be performed.

Despite all these constraints related to the insufficiency of the reagents which did not allow to make several observations, you will find in the following paragraphs, some statistical results obtained.

Results and Discussion

Substrate Composting and Temperature Evolution

Fig 1 shows us the evolution of temperature during composting. The figure shows that the temperature first rose to a certain peak and then decreased thereafter to return to around the original temperature. This situation informs us that the activity of the fermenting bacteria of the substrate increased initially but at a certain moment, the bacteria came up against the effect of the temperature and were gradually eliminated. These are psychrophilic bacteria whose optimum growth temperature is between 0-15°C, mesophilic bacteria whose optimum growth temperature is close to the temperature of the human body, i.e. 37°C and some of the thermophiles whose Growth temperature is between 45°C-70°C.

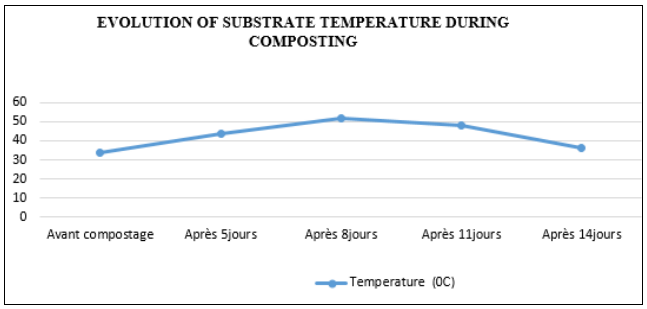

Fig 1: Evolution of temperature during composting

Microbiological Analyzes

Microbiological analyzes were carried out with the aim of qualitatively and quantitatively evaluating the microorganisms (bacteria and molds) present in the substrate during composting. To do this, a control corresponding to T0 (time before composting) was analyzed.

Fig 2 shows us the different types of microorganisms present in the substrate at the start and their quantitative evolution during composting.

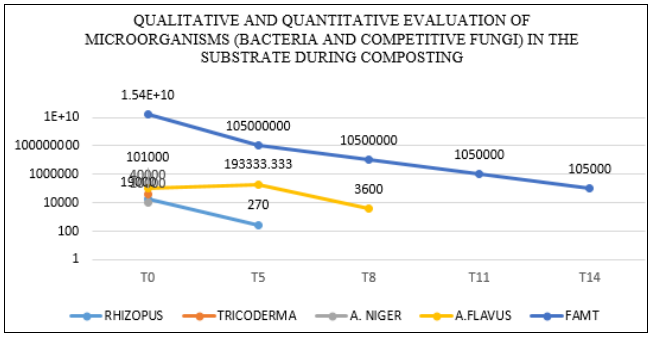

Fig 2: Qualitative and quantitative evaluation of microorganisms (bacteria and competing fungi) in the substrate during composting

The curves in the graph show that the more the composting time increases, the content of microorganisms decreases, even disappearing for some. This is the case with mushrooms which disappear after 8 days of composting. Only the bacteria remain present until the 14th day of composting. This is normal because in a composting process, the rise in temperature leads to the selective elimination of microorganisms that are less resistant to temperature and the subsistence of thermophilic bacteria or their sporulated form.

Table 1: Shows us the level of correlation between temperature and the evolution of bacteria.

Table 2: Shows us the level of correlation between the duration of composting and the evolution of bacteria.

Table 3: Shows us the level of correlation between the duration of composting and the evolution of moulds.

Table 4: Shows us the level of correlation between composting temperature and mold growth.

Table 1: Correlation between temperature and the evolution of bacteria

		Temperature Variation	Average FAMT content
Temperature Variation	Pearson correlation	1	-,639
	sig. (bilateral)		,245
	Sum of squares and cross products	236,800	-134975067191,884
	Covariance	59,200	-33743766797,971
	N	5	5
Average FAMT content	Pearson correlation	-,639	1
	sig. (bilateral)	,245	
	Sum of squares and cross products	-134975067191,884	188185281133116500000,000
	Covariance	-33743766797,971	47046320283279120000,000
	N	5	5

Table 2: Correlation between composting time and bacterial development

		Composting time	Mean FAMT content
Composting time	Pearson correlation	1	-,788
	sig. (bilateral)		,113
	Sum of squares and cross products	117,200	-117053074397,078
	Covariance	29,300	-29263268599,270
	N	5	5
Mean FAMT content	Pearson correlation	-,788	1
	sig. (bilateral)	,113	
	Sum of squares and cross products	-117053074397,078	188185281133116500000,000
	Covariance	-29263268599,270	47046320283279120000,000
	N	5	5

The data in Table 2 show that the correlation between temperature or composting time and bacterial evolution reaches -0.636 which is a negative value, proving that the 2 variables evolve in opposite directions. This confirms the hypothesis that when the temperature increases, the bacteria content decreases.

Table 3: Correlation between composting time and mould growth (SM)

		Composting time	Mould content
Composting time	Pearson correlation	1	-,893*
	sig. (bilateral)		,041
	Sum of squares and cross products	117,200	-556782,158
	Covariance	29,300	-139195,540
	N	5	5
Mould content	Pearson correlation	-,893*	1
	sig. (bilateral)	,041	
	Sum of squares and cross products	-556782,158	3314657135,951
	Covariance	-139195,540	828664283,988
	N	5	5

*. The correlation is significant at the 0.05 level (two-tailed).

Table 4: Correlation between temperature and mould growth(SM)

		Temperature variation	Mould content
Temperature variation	Pearson correlation	1	-,489
	sig. (bilateral)		,403
	Sum of squares and cross products	236,800	-433039,004
	Covariance	59,200	-108259,751
	N	5	5
Mould content	Pearson correlation	-,489	1
	sig. (bilateral)	,403	
	Sum of squares and cross products	-433039,004	3314657135,951
	Covariance	-108259,751	828664283,988
	N	5	5

As for the bacteria, it appears that the correlation between temperature or composting time and mould evolution reaches -0.893 which is a negative value, which proves that the 2 variables evolve in opposite directions. This confirms the hypothesis that when the temperature increases, the mould content decreases.

Moreover, by adjusting the composting time in terms of number of days, the correlation becomes significant at the 0.05 threshold (0.041).

Physico-Chemical Analyses

The physico-chemical analyses of the substrate were carried out in order to highlight the effect of temperature and composting time on the carbon and nitrogen content of the substrate.

Fig 3: Shows the evolution of the nitrogen content of the substrate during composting.

Fig 4: Shows the evolution of the carbon content of the substrate during composting.

Fig 5: Shows the evolution of the C/N ratio of the substrate during composting.
Table 5: Shows the physico-chemical characteristics of the substrate used.

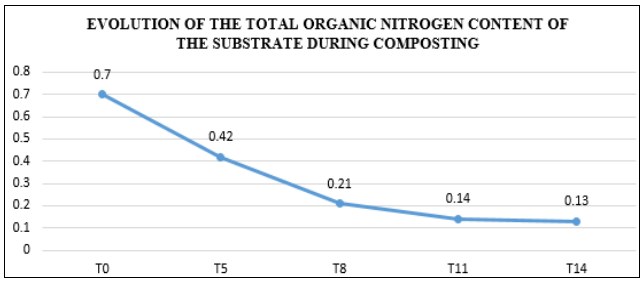

Fig 3: Evolution of the nitrogen content of the substrate during composting

The data in the graph show that as the composting time increases, the nitrogen content of the substrate decreases, first at an accelerated rate until day 8, and then slows down and almost stabilises between days 11 and 14.

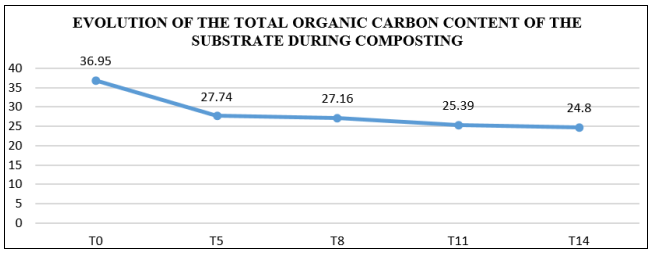

Fig 4: Evolution of the total organic carbon content of the substrate during composting

The data in the graph show that as the composting time increases, the carbon content of the substrate decreases, first with an accelerated rate until day 5, and then slows down and almost stabilises between days 11 and 14, as with the nitrogen content mentioned above.

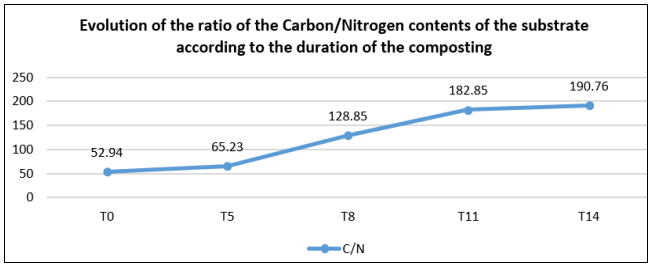

Fig 5: Evolution of the C/N ratio of the substrate during composting

The data in the graph show that the ratio of Carbon to Nitrogen content increases, which proves that the two aggregates decrease at different rates. In our case, it is the nitrogen that decreases at a faster rate than the carbon. This is due to the activity of the fermenting bacteria that consume the nitrogen and carbon materials in the substrate. As a result, there will be a significant increase in the C/N ratio.

Table 5: Physico-chemical characteristics of the substrate used

Parameters / Time (days)	CELL (%)	NTO (%)	C/N
Before composting	37,06	0,70	52,94
After 5 days	27,4	0,42	65,23
After 8 days	27,06	0,21	128,85
After 11 days	25,6	0,14	182,85
After 14 days	24,8	0,13	190,76

CELL=cellulose (carbon); NTO= total organic nitrogen; C/N= carbon-nitrogen ratio
Table 6: Shows the level of correlation between composting temperature and carbon percentage.
Table 7: Shows the level of correlation between composting time and carbon percentage.
Table 8: Shows the level of correlation between composting temperature and nitrogen evolution.
Table 9: Shows the level of correlation between composting time and nitrogen evolution.
Table 10: Shows the level of correlation between the duration of composting and the evolution of the C/N ratio.
Table 11: Shows the level of correlation between the composting temperature and the evolution of the C/N ratio.

Table 6: Correlation between temperature and carbon evolution

		Temperature variation	Percentage of carbon
Temperature variation	Pearson correlation	1	-,518
	sig. (bilateral)		,371
	Sum of squares and cross products	236,800	-78,612
	Covariance	59,200	-19,653
	N	5	5
Percentage of carbon	Pearson correlation	-,518	1
	sig. (bilateral)	,371	
	Sum of squares and cross products	-78,612	97,095
	Covariance	-19,653	24,274
	N	5	5

Table 7: Correlation between composting time and carbon evolution

		Composting time	Percentage of carbon
Composting time	Pearson correlation	1	-,910*
	sig. (bilateral)		,032
	Sum of squares and cross products	117,200	-97,034
	Covariance	29,300	-24,259
	N	5	5
Percentage of carbon	Pearson correlation	-,910*	1
	sig. (bilateral)	,032	
	Sum of squares and cross products	-97,034	97,095
	Covariance	-24,259	24,274
	N	5	5

*. The correlation is significant at the 0.05 level (two-tailed)

Table 8: Correlation between temperature and nitrogen evolution

		Temperature variation	Percentage of nitrogen
Temperature variation	Pearson correlation	1	-,520
	sig. (bilateral)		,369
	Sum of squares and cross products	236,800	-3,880
	Covariance	59,200	-,970
	N	5	5
Percentage of nitrogen	Pearson correlation	-,520	1
	sig. (bilateral)	,369	

	Sum of squares and cross products	-3,880	,235
	Covariance	-,970	,059
	N	5	5

Table 9: Correlation between composting time and nitrogen evolution

		Composting time	Percentage of nitrogen
Composting time	Pearson correlation	1	-,957*
	sig. (bilateral)		,011
	Sum of squares and cross products	117,200	-5,020
	Covariance	29,300	-1,255
	N	5	5
Percentage of nitrogen	Pearson correlation	-,957*	1
	sig. (bilateral)	,011	
	Sum of squares and cross products	-5,020	,235
	Covariance	-1,255	,059
	N	5	5

*. The correlation is significant at the 0.05 level (two-tailed)

The correlation is negative, which shows that the two variables move in opposite directions.

Table 10: Correlation between composting time and C/N ratio evolution

		Composting time	Carbon to Nitrogen ratio
Composting time	Pearson correlation	1	,948*
	sig. (bilateral)		,014
	Sum of squares and cross products	117,200	1329,952
	Covariance	29,300	332,488
	N	5	5
Carbon to Nitrogen ratio	Pearson correlation	,948*	1
	sig. (bilateral)	,014	
	Sum of squares and cross products	1329,952	16807,676
	Covariance	332,488	4201,919
	N	5	5

*. The correlation is significant at the 0.05 level (two-tailed)

Table 11: Correlation between temperature and the evolution of the C/N ratio

		Temperature variation	Carbon to Nitrogen ratio
Temperature variation	Pearson correlation	1	,224
	sig. (bilateral)		,717
	Sum of squares and cross products	236,800	447,876
	Covariance	59,200	111,969
	N	5	5
Carbon to Nitrogen ratio	Pearson correlation	,224	1
	sig. (bilateral)	,717	
	Sum of squares and cross products	447,876	16807,676
	Covariance	111,969	4201,919
	N	5	5

Conclusion

The objective of this study was to demonstrate the effect of composting on the quality of the substrate, on mycelial growth and on the yield of the mushrooms in the case of Pleurotus Ostreatus Var 2175 produced on composted cottonseed chips and Grevillea sawdust respectively. The ultimate aim was to combat contamination, which continues to occur in myciculture despite the fact that pasteurisation has been carried out well.

The hypotheses posed were to verify whether the composting of the substrate makes it possible to create a selective environment that favours mycelial growth to the detriment of competitive and harmful bacteria and fungi, to determine whether composting improves the yield of the fungi, which will enable us to find other applications for this compost that have a higher value and are of great use to society, and to have sufficient quantities available, through the improvement of the yield of the fungi, to increasingly improve the nutritional health of the populations.

The results obtained showed that during composting, the temperature rises in the substrate, thus selectively destroying the microorganisms (bacteria and competing fungi) according to their temperature resistance.

Microbiological analyses were carried out to identify the types of microorganisms and the process of their elimination during composting and these analyses showed that at 11 days of composting, the majority of the microorganisms are already eliminated and this proves the eliminatory effect of temperature on these microorganisms.

Physico-chemical analyses were carried out to check for possible repercussions of the composting process on the nitrogenous and carbonaceous matter of the substrate. The analyses showed that during the composting process, the nitrogen and carbon content decreases significantly, which gradually depletes the substrate of nitrogen and carbon. The carbon/nitrogen ratio increases significantly and this calls for the supplementation of our substrate with nitrogen-rich supplements.

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