643

European J.Appl.Microbiol. 1,327-335(1975)

Influence of CO_2 Concentration on the Mycelium Growth of Three Pleurotus Species

F. ZADRAŽIL
Research Institute von Sengbusch, Hamburg

Received August 14, 1974; Final Version July 2, 1975

Summary

1. Increased CO_2 concentration in the air has a positive effect on mycelium growth in the cultivation of *Pleurotus ostreatus, P. florida* and *P. eryngii* (Agar cultures).
2. Between 16% and 22% CO_2 in the air is the optimum for the growth of the 3 above-mentioned Pleurotus species.
3. Concentrations of 36 vol.% CO_2 inhibited growth of all 3 species.
4. Both sterile and pasteurized straw substrata showed 20-25% (of vol.) CO_2 3 days after inoculation. Mycelium growth was normal. CO_2 concentration stands in relation to the amount of inoculation material, temperature, composition of substratum, and other environmental factors.
5. The stimulating effect of CO_2 on the growth of the Pleurotus species studied and their high tolerance to CO_2 have a special importance in Pleurotus farming.
6. Under semianaerobic conditions with high CO_2 concentration in the gaseous phase of substratum, the competitive microorganisms are eliminated and Pleurotus mycelium can grow well in the non-sterile substratum.

Carbon dioxide belongs to the most important ecological factors. The quantity of CO_2 in the ecosystem determines biological activity and the quantity of micro-organism (Ahrens & Thalmann, 1970; Zadražil, 1971, etc.). On the contrary one can consider the differing concentrations of CO_2 in the ecosystem as a selective factor in the range of species of micro-organisms (MacFadyen, 1973; Zadražil, 1973a).

The CO_2 requirement of bacteria, yeasts and moulds has long been recognized (Valley & Rettger, 1927; Hermann, 1963; Bronn & Lucà, 1973). Removal of CO_2 from the ecosystem can cause decrease of growth (Lafferty, 1963). The optimum CO_2 concentration varies for each type of microorganism.

In 1971, Walsh & Stewart proved exchanges between oxygen, carbon dioxide and the cellulolytical activity in some kinds of fungi. In 1974, Schánĕl found a direct relationship between the CO_2 concentration and the exoenzymatic activity of wood-destroying fungi.

The influence of CO_2 on the mycelium growth and the fruit body building of the basidiomycetes was mainly studied with the *Agaricus bisporus* (white mushroom). In 1958, 1959 and 1972, Tschierpe determined that at 2% CO_2 and above, the mycelium of *Agaricus bisporus* was inhibited in growth; at a concentration above 32 vol.%, the growth stopped.

Rast & Bachofen (1965, 1967a, b) determined that the mycelium of *Agaricus bisporus* is able to fixate CO_2. San Antonio & Thomas (1972, 1973) specified the optimum concentration for the *A. bisporus* as between 0.1% and 0.5% CO_2.

In 1954 and 1956, Plunkett also recognized CO_2 as the most important factor of the fruit body building of *Collybia velutipes* and in 1963 Niederpruem studied the same with the *Schizophyllum commune*.

The influence of CO_2 on the growth of wood-destroying fungi in connection with wood protection was studied by Lambert (1933), Zycha (1937), Plunkett (1954, 1956), Gundersen (1961), Rypáček (1966), Jensen (1967) and Schánĕl (1970). As in the above-mentioned papers little information is given about the influence of CO_2 on the Pleurotus species, we studied in the following the effect of different concentrations of carbon dioxide under various conditions as a basis for the biotechnology of Pleurotus production.

METHODS

Cultures of fungi:

Pleurotus florida (FovoSe) - selected culture P1 own breeding of *Pleurotus florida* (Eger, 1965)

Pleurotus ostreatus (Jacq. ex Fr.) Kummer - provenance Bäarn, culture P4

Pleurotus eryngii (DC. ex Fr.) - own isolation - Spain, culture P5

Experiment No.1

Growth of Mycelium on Agar-Plates with Different Concentrations of CO_2.
Cultures in petri dishes were cultivated at 25°C in incubators with different contents of CO_2. Normal air (0.03% CO_2) was used as control.

One half of the petri dishes inoculated in the center with a round inoculum of 7 mm diameter each were placed into a normal incubator at 25°C; the other half of the petri dishes was put into an incubator (Heraeus KB 600 K-CO_2) under a controllable and constant CO_2 concentration.

At the following concentrations of CO_2 in the air, the Pleurotus species were incubated: 5.0, 10.0, 16.6, 22.0, 28.8 and 37.5 vol.%. During the period of the experiment, the difference of the CO_2 concentration was approximately ± 0.5%.

Analysis of the Experiments. The radial mycelium growth was measured in regular time intervals. The velocity of the mycelium growth (diameter of culture and time) was discovered as a linear correlation for 5 days. For each CO_2 concentration we made 3 to 5 experiments, repeating each one four times. The number of measurements in Table 1 are shown under n.

The Composition of Nutrient Media. Malt extract 5 g, soy bean flour 10 g, pepton 1 g, KH_2PO_4 0.5 g, $MgSO_4.7H_2O$ 0.5g, $FeCl_3$ (1% solution) 1 ml, yeast extract 0.1 g, agar 15 g, 1 l H_2O, pH 5.2.

Experiment No.2

Submerged Culture. Submerged culture on the shake apparatus (500 ml Erlenmeyer flask with 200 ml nutrient media) was inoculated with Pleurotus mycelium (3 agar mycelium pieces of 7 mm Ø). For gas addition, we used the following concentration of CO_2: 5, 10 and 15 vol.% of CO_2 in air). The mycelium growth was finished after 5 or 9 days and the dry weight and the pH value of nutrient media were determined.

Experiment No.3

Production Experiment. In order to determine the CO_2 production in sterile and pasteurized substratum (90°C, 1 h) we made mycelium growth experiments. In 1 l jars 0.5 kg of straw substratum was sterilized (Zadražil, 1973b) and inoculated with 3 agar pieces of mycelium (Ø 7 mm). The CO_2 concentration in the substratum was measured with the Dräger-Gasspürgerät type 19/31.

RESULTS

The results showing the effect of CO_2 on the growth of mycelium (Experiment No.1) are shown in Table 1 and Fig.1.

Table 1
Effect of carbon dioxide on the radial mycelium growth (mm in 5 days) of *P. ostreatus, P. florida and P. eryngii*
(n = number of measurements)

Vol.% CO_2 in the air	*P. ostreatus*		*P. florida*		*P. eryngii*	
	in mm	n	in mm	n	in mm	n
5%	61.9	20	56.6	20	39.3	30
Control (0.03)	58.4	20	48.1	30	34.9	48
10%	55.7	46	55.4	52	33.4	84
Control (0.03)	48.5	52	51.4	52	29.4	88
16.6%	61.5	88	60.4	80	33.3	104
Control (0.03)	49.1	104	52.2	112	30.3	104
22.0%	56.7	150	54.5	150	25.4	220
Control (0.03)	53.0	170	49.9	180	19.4	220
28.8%	57.2	136	59.2	144	28.7	304
Control (0.03)	54.3	160	55.4	152	30.9	288
37.5%	28.7	104	31.3	232	17.3	136
Control (0.03)	44.9	160	51.5	128	29.8	248

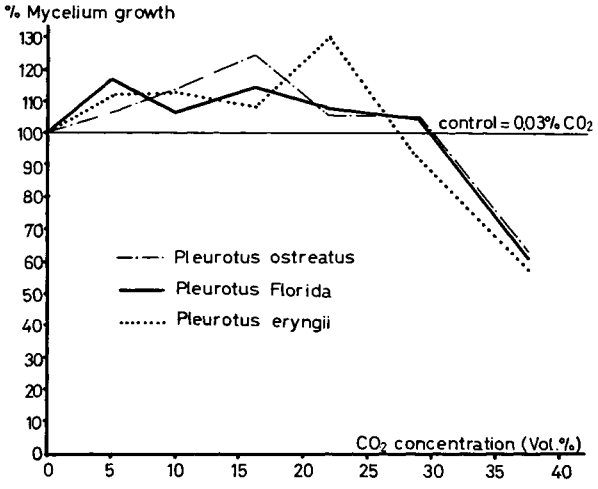

Fig.1
Effect of carbon dioxide on the mycelium growth of *Pleurotus ostreatus, P. florida* and *P. eryngii* (control -0.03% CO_2 = 100% Mycelium growth)

It may be observed that a CO_2 concentration in the air of up to 28 vol.% vol. stimulates the growth of *Pleurotus ostreatus* and *P. florida*. *P. eryngii* is the exception with a limit for stimulation of about 22%, by 28% CO_2 mycelium is inhibited. The highest concentration during our studies of 37.5 vol.% reduced mycelium growth in all three Pleurotus species by about 40% as compared with a control at 0.03% CO_2. (see Table 1 and Fig. 1)

Table 2
Effect of CO_2 concentration on the mycelium growth (6 repeats/experiments) in submerged culture (*P. florida*, strain P1)

	5-day growth mycelium weight in mg	pH of culture at end of experiment	9-day growth mg	pH
Gas with air control	-	-	576.4 ±100.0	5.3 ±0.21
Air + 5% CO_2	299.3 ± 80.0	5.9 ±0.08	673.0 ± 80.0	5.8 ±0.14
Air + 10% CO_2	333.5 ± 50.0	5.7 ±0.01	778.9 ±150.0	5.8 ±0.15
Air + 15% CO_2	349.8 ± 40.0	5.6 ±0.08	766.9 ±150.0	5.8 ±0.13

Similar results with a stimulating effect of CO_2 on the submerged cultures, Experiment No.2 (Table 2), were noticed.

The influence of CO_2 on mycelium growth remained in the same sequence in all the experiments. With increasing CO_2 concentration in the air, also the mycelium weight of the various cultures increased. But the average differences between the repetitions were bigger than the difference between the variants of the experiments. In this case, one can only speak of a tendency. The pH changes by the addition of gas to the submerged culture show quite an insecure correlation to the mycelium growth.

In order to prove that the Pleurotus mycelium grows under practical conditions at high concentrations of CO_2, the CO_2 content in inoculated substrata (sterile and pasteurized) was measured. The air was analyzed at regular intervals, the results being shown as Fig.2. In order to test the activity of various mycelium masses, two quantities of inoculation material were introduced into the substratum. Three days after inoculation, the CO_2 concentration in both cases had already risen to over 20%. As excepted, the case with 10% inoculum showed a greater concentration than the case with 5%. 4 and 6 days respectively after inoculation, CO_2 concentration had reached a maximum, then decreased, finally showing no material alteration from the 13th to the 31st day. After 13 days the substratum was well permeated.

In a temperature experiment (mycelium growth at 25 and 30°C) with 1‰ inoculation material, the CO_2 concentration climbed slowly and continuously, reaching a maximum after 20 days, at 25°C this was 30% and at 30°C, 35% CO_2 (Fig.3). After this

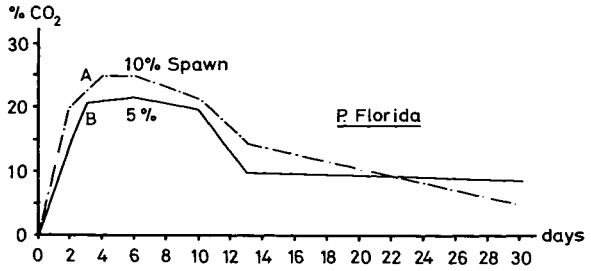

Fig.2
Effect of spawn quantity (*Pleurotus florida*) on CO_2 concentration in wheat straw during the mycelium growth.
A. 10% spawn
B. 5% spawn

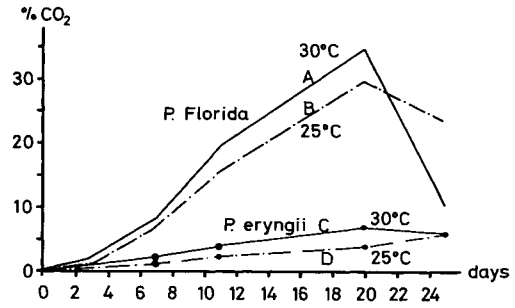

Fig.3.
Effect of temperature on CO_2 concentration in wheat straw substratum during the mycelium growth (1%. spawn)
A. *Pleurotus florida* 30°C
B. *Pleurotus florida* 25°C
C. *Pleurotus eryngii* 30°C
D. *Pleurotus eryngii* 25°C

time, the CO_2 concentration declined. In the 30°C system the decline was more rapid than in the 25°C. Similar temperature effects were noted with *P. eryngii*, but with lower CO_2 concentration and slower growth. Here the CO_2 did not reach the optimum concentration for growth.

The high tolerance of Pleurotus species to CO_2 is demonstrated by this example, and the results could be reproduced in differently organized experiments. Carbon dioxide production is nevertheless dependent on the ingredients in the substratum (unpublished).

DISCUSSION

From the results the stimulating effect of CO_2 on the mycelium growth of 3 Pleurotus species is evident. With an increase of the CO_2 concentration from 0.03% to 16%, the mycelium growth of both *P. ostreatus* and *P. florida* was stimulated. *P. eryngii* still showed a positive result at 22.0% CO_2 and a growth decrease at 28% CO_2. *P. ostreatus* and *P. florida* were inhibited at a higher concentration.

By repeating various experiments, the results were reproduceable and in agreement with those of Schánél (1970). He

determined that *P. ostreatus* shows the best growth at 10 vol.% CO_2 (120.4%), only 108% at 20% CO_2, and at 30% CO_2 a depression of 83% against the control level (100%).

Under different conditions in submerged culture similar increases of mycelium growth were shown. 5%, 10% and 15% CO_2 in the air increase the yield of mycelium. On aeration with normal air the results were partly positive, partly negative compared to nonaerated cultures and those supplied with CO_2, respectively. The results of the daily growth (mg/day) did not correspond as much as in the case of agar cultures. The different results can be explained with the special behavior of Basidiomycetes in submerged culture. The mycelium growth depends on the special conditions of cultivation. We obtained different types of growth (secondary spore production) and reproduction rates in an experiment with Pleurotus and other Basidiomycetes under similar conditions. The germination and growth of secondary spores have most probably influenced the yield of mycelium in the submerged culture.

We could also prove the special tolerance of the Pleurotus mycelium toward high CO_2 concentrations both in sterile and pasteurized straw substratum. 3 days after the inoculation there was a CO_2 concentration of more than 20%; the mycelium developed quickly and normally. In a closed system, the CO_2 concentration reached 35%. The high resistance of Pleurotus and the stimulating effect of CO_2 on the growth of Pleurotus mycelium are the most important bio-technical factors in industrial Pleurotus production.

The mycelium growth develops under semianaerobic conditions with a high content of CO_2 in the gaseous part of the substratum. This fact does not exclude the necessity of oxygen for the mycelium growth. In 1974, Zadražil determined the relation between mycelium growth and the decrease of oxygen in the nutrient media. The high concentration of CO_2 in the substratum not only has a stimulating effect on the mycelium growth of the Pleurotus species but also an inhibitive effect on other species in the non-sterile straw substratum (see Zadražil, 1973).

Pleurotus cultures (pasteurized substratum which have grown under semianaerobic conditions were free from other aerobic organism. On the other hand, those cultures which have grown under the same conditions, but fully aerobic, were, apart from Pleurotus mycelium, occupied by "weed fungi".

In this case the CO_2 is the most important factor in Pleurotus culture. With a high concentration of CO_2, the ability of reproduction of the production is secured and saves the high expenditure of work of the sterile technique.

In mass production of mycelium on solid media, the contents

of CO_2 could be regulated from the beginning of cultivation by adding and circulating CO_2-enriched air. Thus the temperature of CO_2 and O_2 could be regulated and kept at an optimum level during the cultivation.

The fruit body building of Pleurotus species in comparison to the period of mycelium growth is a real aerobic process. The decomposition of the cellulose-lignin-complex is directly related to the exchange of air (Zadražil, 1974). Under aerobic conditions, the substratum was decomposed more rapidly than under semianaerobic conditions. In 1974, Zadražil summarized the complex of cultivating conditions of Pleurotus production.

The experiments regarding CO_2 fixation of Pleurotus species will be continued.

Acknowledgement. We would like to offer our thanks to the Deutsche Forschungsgemeinschaft for their support of our studies. We further wish to thank Mrs. Marianne Schneidereit for her assistance in these experiments.

REFERENCES

Ahrens,E., Thalmann,A.: (1970). Z.Pflanzenernähr.Düng.Bodenkunde 127, 143-154

Bronn,W.K., Lucà,S.: (1973). 3.Symposium Technische Mikrobiologie, Berlin

Eger,G.: (1965). Arch.Mikrobiol. 50, 343-356

MacFadyen,A.: (1973). Pedobiologia 13, 140-149

Gundersen,K.: (1961). Nature 190, 649

Hermann,D.: (1963). Beij.Arch.Mikrobiol. 45, 373-397

Jensen,K.F.: (1967). Forest Sci. 13, 384-389

Lafferty,R.M.: (1963). Arch.Mikrobiol. 44, 373-405

Lambert,E.B.: (1933). J.Agr.Res. 47, 599-608

Niederpruem,D.J.: (1963). J.Bact. 85, 1300-1308

Plunkett,B.E.: (1954). VIII Congr.Int.Bot., Paris, Rapp. and Comm.Sect. 18-20. p. 101-102

Plunkett,B.E.: (1956). Ann.Bot. (London) N.S. 20, 563-586

Rast,D., Bachofen,R.: (1965). Verhandlungen der Schweiz. Naturforschenden Gesellschaft, p. 125-129

Rast,D., Bachofen,R.: (1967a). Arch.Mikrobiol. 57, 392-405

Rast,D., Bachofen,R.: (1967b). Arch.Mikrobiol. 58, 339-356

Rypáček,V.: (1966). Biologie holzzerstörender Pilze. Jena: VEB. G. Fischer

San Antonio,J.P., Thomas,R.: (1972). Mushroom Science 8. 623-629

San Antonio,J.P., Thomas,R.: (1973). Mushroom News, Vol. 21, Nr. 12 18-22

Schâněl,L.: (1970). Folia Biologia XI, 27, 67-72

Schâněl,L.: (1974). Česká Mykologie, 28, 113

Tschierpe,H.J.: (1958). Diss., Techn. Universität Berlin

Tschierpe,H.J.: (1959). Mushroom Science IV, 211-221 Kopenhagen
Tschierpe,H.J.: (1972). Mushroom Science VIII, 553-591 London
Tschierpe,H.J.: (1958). Die Bedeutung des Kohlendioxyds für den Kulturchampignon. Diss., Techn. Universität Berlin
Valley,G., Rettger,L.F.: (1927). J.Bact. 14, 101-137
Walsh,J.H., Stewart,C.S.: (1971). Trans.Br.Mycol.Soc. 57, 75-84
Zadražil,F.: (1971). Der Einfluß von langjährigen Düngungsmaßnahmen mit Stroh- und Gründüngung sowie Stickstoffdüngung auf einige physikalische, chemische und biologische Bodeneigenschaften. Diss., Universität Gießen, Landw. Fakultät
Zadražil,F., Schneidereit,M.: (1972). Der Champignon 135, 25-32
Zadražil,F.: (1973a). Bericht der DFG. (unpublished)
Zadražil,F.: (1973b). Der Champignon 139, 3-4
Zadražil,F.: (1974 in print). Mushroom Science IX, Tokio
Zycha, H.: (1937). Zbl. Bakt. II Abt. 97, 222-224

Dr. Frantisek Zadražil, Forschungsstelle von Sengbusch GmbH,
D-2000 Hamburg 67, Waldredder 4 , Federal Republic of Germany