582.287.238:581.1.035.7

MEDEDELINGEN LANDBOUWHOGESCHOOL
WAGENINGEN • NEDERLAND • 74-25 (1974)

SOME NOTES ON THE PHYSIOLOGY OF A LUMINOUS FUNGUS

E. C. WASSINK

Laboratory of Plant Physiological Research,
Agricultural University, Wageningen, The Netherlands

(Received 13-XII-1974)

364th Communication (4th on Fungus luminescence)*

1. *Introduction*

The large majority of luminous fungi belong to the white-spore lamellous Basidiomycetes and some closely related porous ones. Luminescence of mycelia is far more common than that of the fruit-bodies. Luminescence of fruit bodies is found in a fair number of tropical species. In Europe there is only one such species, viz., *Pleurotus olearius* in the South. In Holland it has been found a few times on oak trees. Luminescence of mycelia occurs in quite a few common European species, e.g. *Armillaria mellea*, and particularly in the genus *Mycena* and closely related *Omphalia* species. Natural phenomena produced by luminous fungi are e.g. luminous wood (mostly by *Armillaria mellea*) and luminous leaves (probably mostly due to small species of *Mycena* or *Omphalia*) (1).

The isolation of luminous fungi is easy. From not too badly rotten wood the luminous fungus can be isolated on cherry agar; the procedure should be performed in tubes, not on dishes which are too liable to pollution by rapidly growing Imperfects; most species can also easily be obtained from aseptically collected spores (1).

The fungi can be grown and propagated on e.g. cherry extract and bread extract agar in tubes. More abundant growth under conditions suitable for the physiological approach can be obtained in submerged shake cultures in which the mycelium develops as submerged spheres, e.g. in cherry or bread crumb extracts. Luminous fungi that grow well in this way are, e.g., *Armillaria mellea*, *Mycena polygramma*, and *Omphalia flavida*, now mostly called *Mycena citricolor*. In the work to be reported here, *Omphalia flavida* was used throughout. Fungus luminescence may be denoted as weak to very weak in most cases; nevertheless, by long exposures, a luminescent *Omphalia flavida* culture may be photographed in its own light on a shaking machine (2).

* 1st: ref. (1); 2nd: ref. (2), 3rd: ref. (7).

2. *Culture methods*

In persuing our previous work, we aimed at studying possible relations between growth, respiration, and luminescence of material grown in shake cultures. Therefore, a culture method had to be developed in which a defined energy source could be applied for the sake of the respiration measurements, and which should be as clear as possible for the sake of the luminescence measurements. Without further additions, the fungus does not grow on a simple sugar/inorganic salt medium.

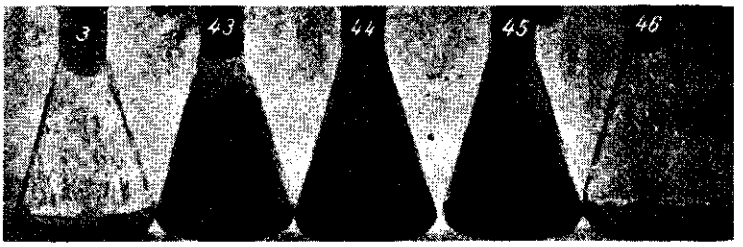


FIG. 1. Effect of yeast extract on the assimilation of glucose by *Phycomyces* (adapted from ref. 3).
Flask 3: mineral solution + 1 ccm yeast extract 3c, no glucose
Flask 43: as flask 3, + 4% glucose
Flask 44: as 43, however, with 1/5 ccm yeast extract
Flask 45: as 43, however, with 1/25 ccm yeast extract
Flask 46: as 43, however, no yeast extract.
For further details, see ref. 3, Table X.

The problem was solved satisfactorily in the same way as practiced over 40 years ago by the author for the cultivation of the Phycomycete *Phycomyces* on a defined energy source for respiration studies (3), see also fig. 1. In both cases a simple inorganic salt solution with glucose, supplied with a small addition of purified yeast extract which as such had no appreciable nutrient value, provided excellent growth. The same purification procedure as in the case of *Phycomyces* (4) was followed, and the first two steps yielded the best results with *Omphalia*. In the case of *Phycomyces*, it has been found elsewhere that thiamin, vitamin B1, can replace the yeast extract (5). For *Omphalia*, thiamin in most cases yielded about half the dry wt. production enabled by the yeast extract additions. A small dose of a Difco yeast preparation has about the same effect as the yeast extracts prepared according to the prescription for *Phycomyces* (fig. 2 which also contains some further details). A combination of certain factors present in the Difco yeast preparation in combination with thiamin (which is also present in Difco) did not yield dw. productions beyond those obtained with thiamin alone.

3. *Kinetics of growth, sugar consumption and acidification*

The following figures show some kinetics of dry weight increase, sugar decrease, pH and $[H^+]$ during the growth of glucose-yeast and glucose-thiamin

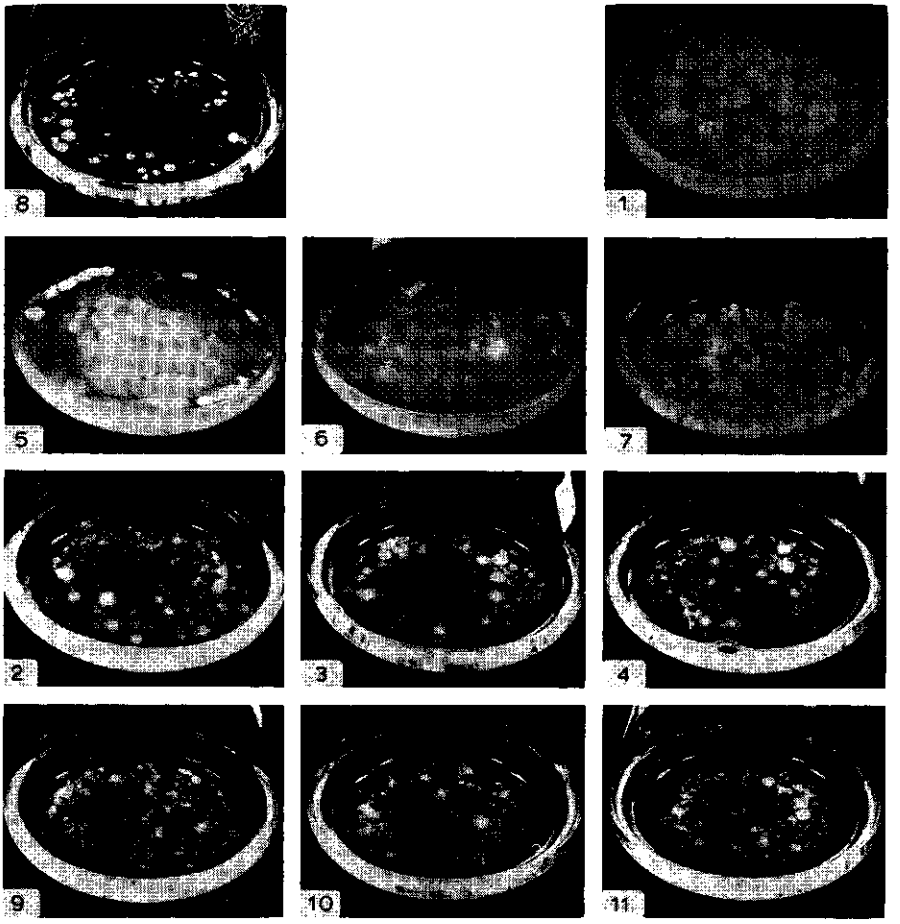


FIG. 2A Growth of *Omphalia flavida* in shake cultures on glucose-salt solution with vitamin additions (Experiment I, started 15-IX-1971, harvested 12-X-1971).
I: Photographs of cultures (12-X-1971).

Flask	Glucose 1%	Thiamin 10^{-6}Mol	Yeast extract fraction
8	+	—	—
5	+	+	1C
2	—	+	1C
9	—	—	1C
6	+	+	2B
3	—	+	2B
10	—	—	2B
1	+	+	—
7	+	+	3C
4	—	+	3C
11	—	—	3C

Yeast extracts: 300 ml from 350 g baker's yeast (→ fraction 1C). Fractions, cf. ref. (4); 4 ml of each fraction added to 150 ml culture medium (= 1 flask). Dilution of all fractions the same as Fraction 1C.

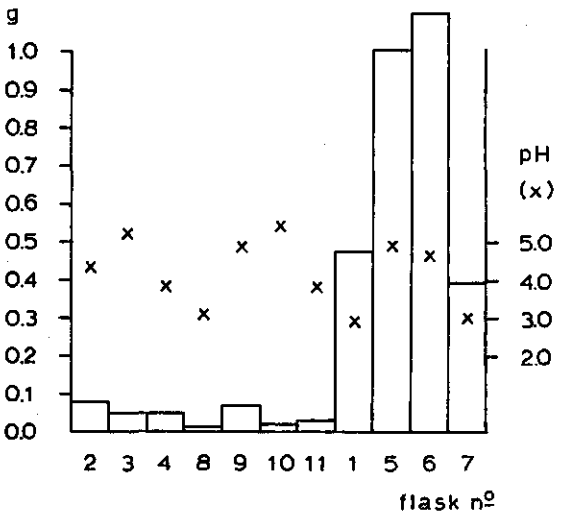


FIG. 2AII. Dry wt. (g) and pH-values at harvest.

cultures, each as averages of 4 experiments. The cultures were grown in 750 ml flasks with 150 ml medium, for about 3 weeks. $[H^+]$ has been calculated, realizing that $pH = -\log [H^+]$ and looking up $[H^+]$ after due conversion of -pH, in a logarithm table.

Fig. 3 shows that increase in dry wt. in a glucose-yeast culture ceases rather abruptly after $9\frac{1}{2}$ days, dw. remains constant for ca. 7 days and then slightly decreases. Comparison of glucose decrease in the medium with dry wt. production is facilitated by considering the inverted glucose decrease curve. In the beginning glucose decrease parallels dw production, but continues rather similarly until exhaustion. Sugar consumption in this phase obviously can only be accounted for by respiration of the fungus.

$[H^+]$ increase also parallels fairly well dw. production but continues somewhat longer, then comes to a standstill, and starts to decrease, practically at the moment glucose is exhausted. The conclusion is tempting that some (mixture of) organic acid(s) is produced by the fungus and metabolized as soon as sugar is exhausted. Certainly, this preliminary conclusion will have to be substantiated further.

However, glucose-yeast cultures fairly consistently show re-increase of pH towards the end of the experiments.

Figs. 4, 5, 6 show daily rates of the various compounds, derived from the slopes in fig. 3. Fig. 4 shows all points, fig. 5 averaging a few big humps, fig. 6 averaging rates over longer growth periods. Figs 4 and 5 substantiate the data from fig. 3, viz., rate of dw. increase dropping from high rates rather suddenly to a value near zero and gradually turning to slightly negative rates. Glucose decrease assumes rates very much paralleling those of dw. increase, but maintaining high rates somewhat longer. $[H^+]$ somewhat belatedly follows the drop

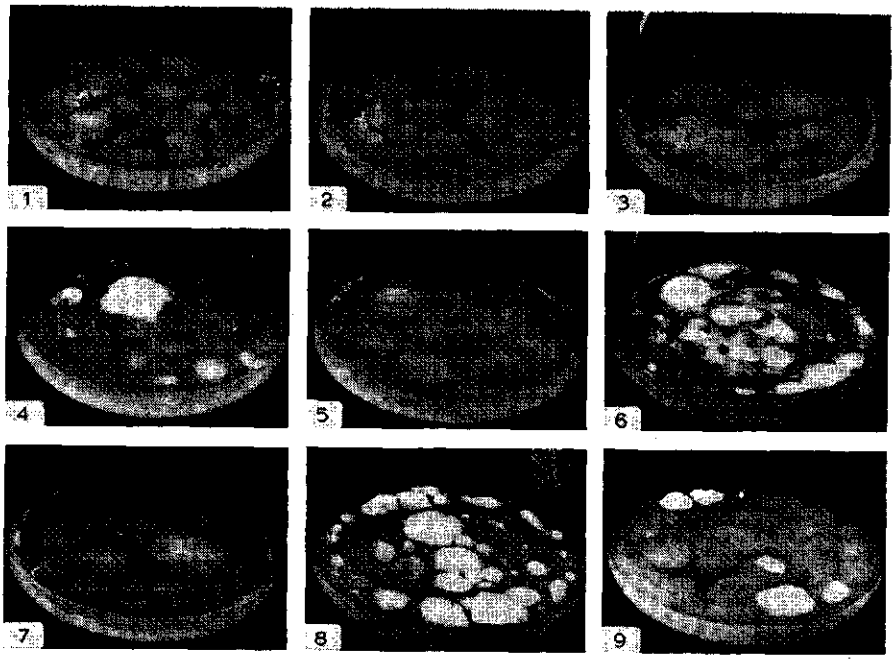
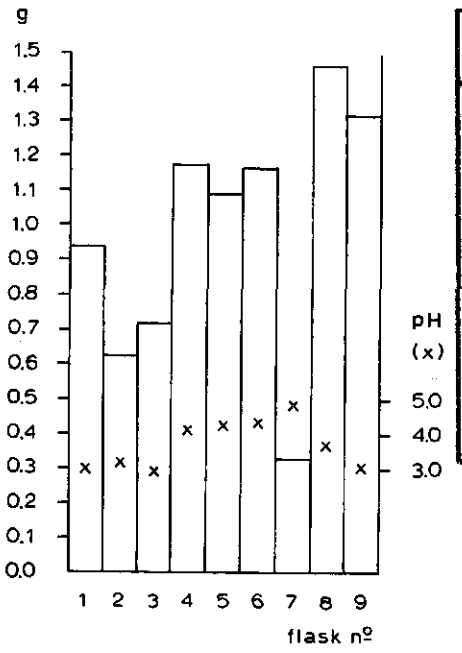


FIG. 2B Growth of *Omphalia flavida* in shake cultures on glucose-salt solution with different concentrations of glucose, thiamin, and Difco yeast preparation (Experiment V, started 12-IV-1972, harvested 3-V-1972).
I: Photographs of cultures (3-V-1972).

Flask	Glucose	Thiamin	Difco yeast preparation
1	1 %	10^{-7}Mol	—
4	1 %	—	0.1 g
7	0.5%	—	0.2 g
2	1 %	10^{-6}Mol	—
5	1 %	—	0.2 g
8	1.5%	—	0.2 g
3	1 %	10^{-5}Mol	—
6	1 %	—	0.3 g
9	2 %	—	0.2 g



Flask	pH	Fresh wt.	Dry wt.
1	3.0	62.029	0.946
2	3.2	56.545	0.630
3	2.95	56.778	0.723
4	4.1	95.658	1.179
5	4.3	83.789	1.098
6	4.45	89.815	1.178
7	4.9	20.215	0.333
8	3.7	101.941	1.464
9	3.1	72.945	1.323

FIG. 2BII (left). FIG.2BIII (right).

II: Dry weights (g) and pH-values at harvest
III: pH, fresh weight and dry weight data at harvest.

in rate of dw. increase and shows strongly negative rates (= decrease) as soon as rates of sugar decrease have become very low.

Fig. 6 shows that, over longer periods, the three phenomena discussed parallel each other, bringing out the differences discussed in the simplest way.

Figs. 7 and 8 show the same for glucose-thiamin cultures. Dw. increase stops at a lower level than in glucose-yeast cultures, but dw. hardly decreases towards the end.

Sugar decrease fairly well parallels dw. production and so does $[H^+]$ increase, but both continue parallelly and more or less at similar rates onto the end of the experiment. One may, again, suggest that the full-grown cultures continue to consume sugar and to produce organic acid(s). Both for glucose-yeast cultures and glucose-thiamin cultures it should be observed that it is not fully certain, as yet, whether the observed acidification of the medium is (in part) due to, e.g. the accumulation of SO_4^{--} ions owing to consumption of NH_4^+ from $(NH_4)_2SO_4$. Especially the changes in acidity, observed in the glucose-yeast cultures, in the later parts of the culture period, however, do not seem to support this possibility.

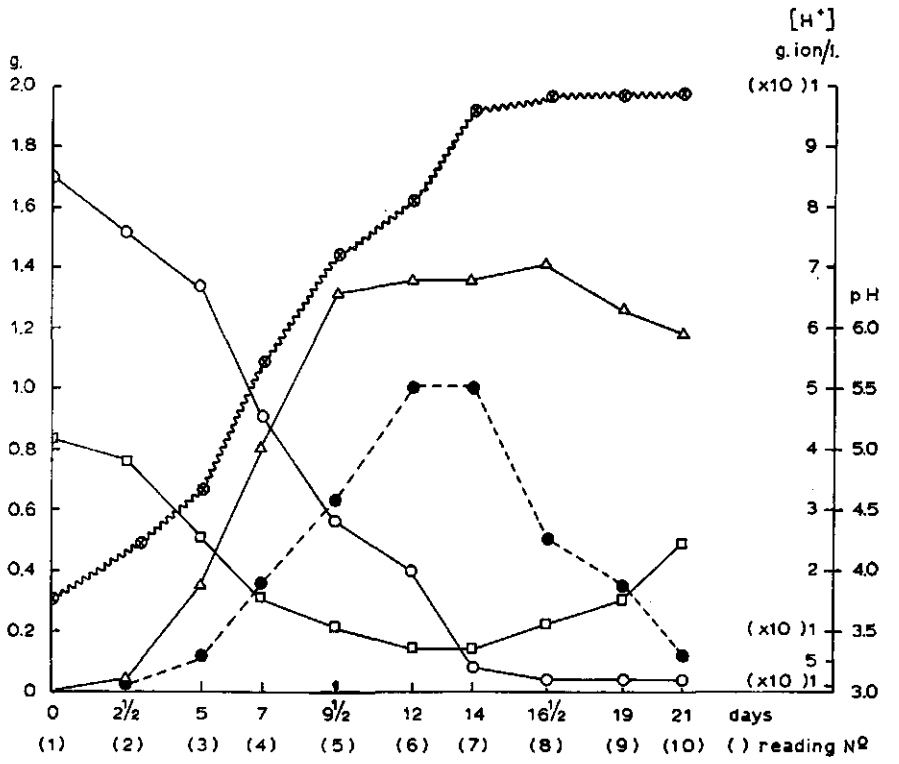


FIG. 3. Glucose-yeast culture.
Dry weight of mycelium in 150 ml medium (△)
Glucose in 150 ml medium (○)
Glucose in 150 ml medium, inverse (⊗)
pH (□), [H⁺] (●), right scales.
] left scale.

Fig. 8, showing the daily rates, illustrates the features discussed in a different way. The dw. increase picture is not much different from the one obtained from glucose-yeast cultures, except the lower maximal rates (by the way, showing that in glucose-yeast cultures as compared with glucose-thiamin cultures not only a higher maximum dw. level is reached but also at higher rates).

Glucose decrease rates remain at high levels for several days. $[H^+]$-production rate shows a remarkable dip around the time the rates of dw. increase suddenly decrease. Since 4 experiments have been averaged, this phenomenon may have some real significance.

The impression had been gained in previous series that the fungus starts vigorous growth only after it has 'succeeded' in bringing pH down to around 4.0.

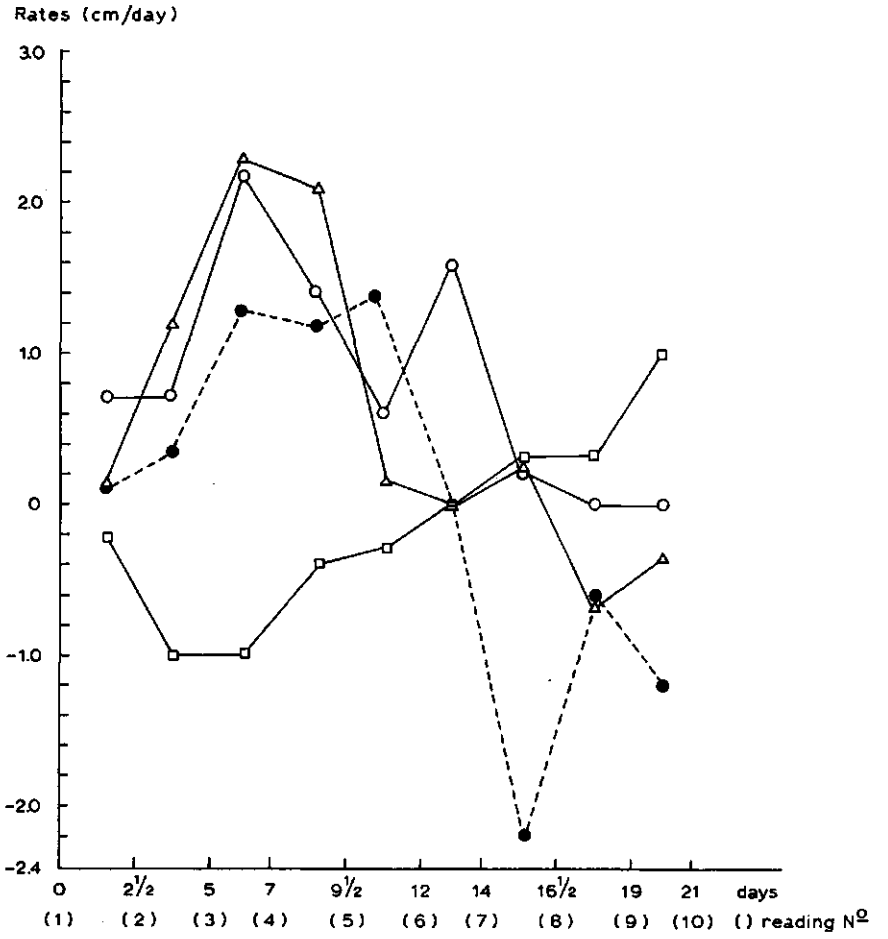


FIG. 4. Glucose-yeast culture.
Rates (cm/day) of dry weight increase (△), glucose decrease in medium (○), change in pH (□) and change in $[H^+]$ (●).
Units: 1 cm = 0.1 g/day (△ and ○), 0.25 pH/day (□), 0.5 × 10^4 gr.ion $[H^+]$/day (●).

Fig. 9 shows four simultaneously run glucose-yeast culture series at different initial pH values, viz. the normal solution, non buffered, containing 0.5% KH_2PO_4 (A), and 3 series buffered with phosphate with initial pH-values of 5.54, 6.47 and 7.17 respectively. The records show dw. produced, H^+ plotted as 'log' (= –pH), and sugar decrease. There is an obvious parallelism between the successive increase in dw. production and the change in log $[H^+]$. Especially the start of vigorous growth only after log $[H^+]$ was brought down to 4.0 in the

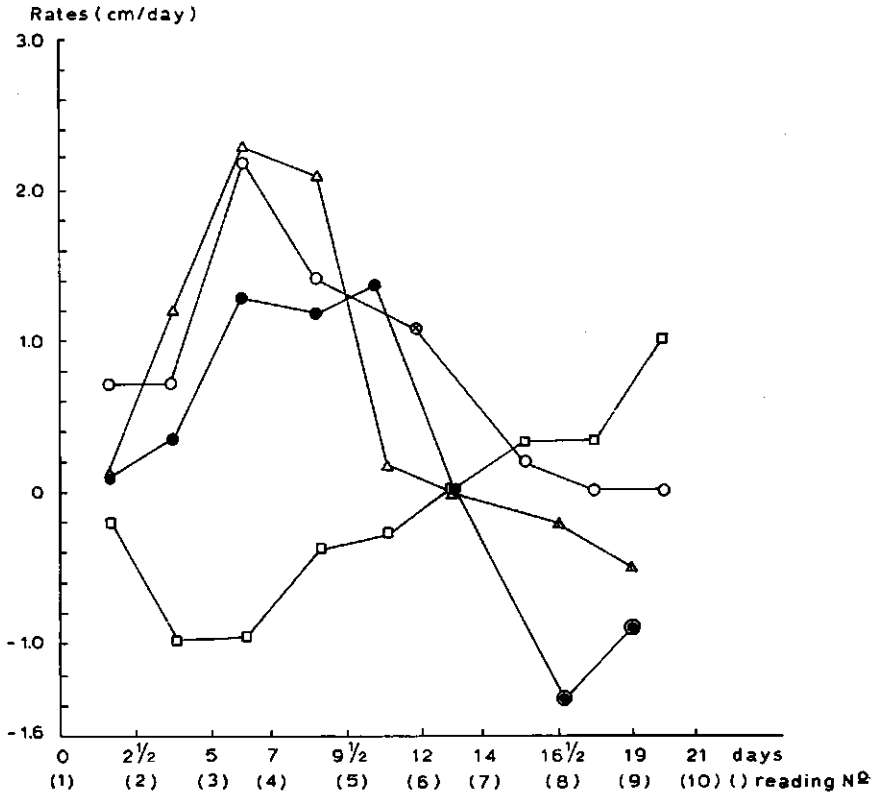


FIG. 5. Glucose-yeast culture.
Rates (cm/day) of dry weight increase (△), glucose decrease in medium (○), change in pH (□) and change in $[H^+]$ (●); ▲ ⊗ ⊙ averages of 2 points.
Units: 1 cm = 0.1 g/day (△ and ○), 0.25 pH/day (□), 0.5×10^{-4} gr.ion $[H^+]$/day (●).

most alkaline solution is striking. Unfortunately, the sugar decrease curves show some irregularities, of which especially the one for the most alkaline solution seems odd. An explanation cannot be traced any more.

In a previous period of our work we have tried, unsuccessfully so far, to bridge the gap between the growth in glucose-thiamin media and in glucose-yeast media. Various amino-acid additions, and also peptone and urea have been tested, with either no, or negative results.

We have available an analysis of the Difco preparation supplied by the manifacturer, listing several amino-acids and a set of 'vitamin factors'. The latter were added to a glucose-thiamin culture in the concentrations indicated and compared with a glucose-thiamin culture as such, and with a glucose-yeast medium. Fig. 10 shows that the addition has not improved final dry wt. produced,

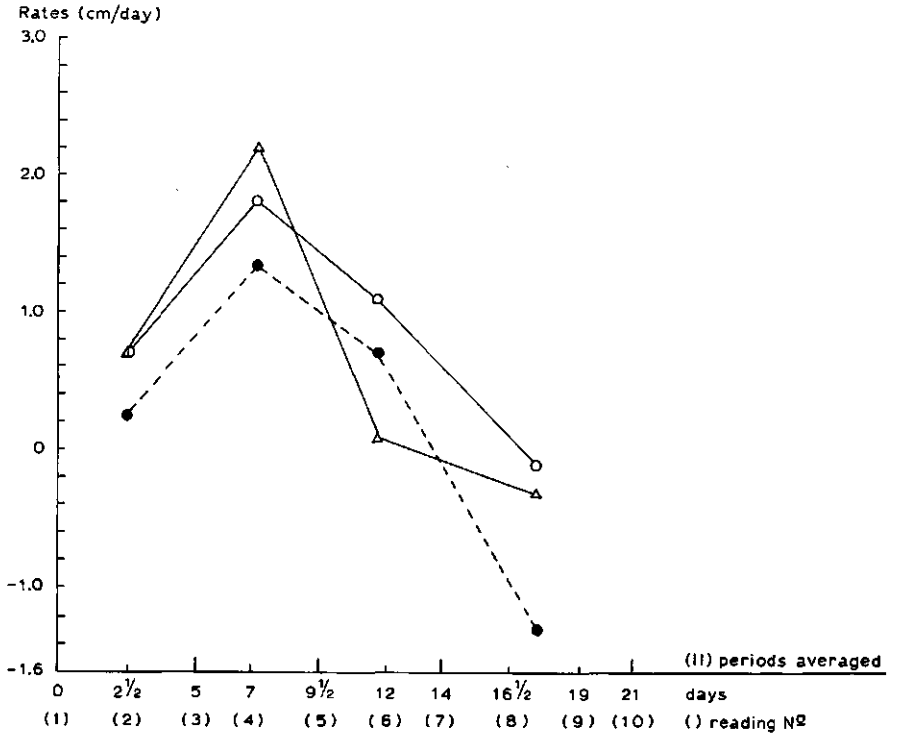


FIG. 6. Glucose-yeast culture.
Rates (cm/day) of dry weight increase (△), glucose decrease in medium (○), and change in $[H^+]$ (●). Averages over several days.
Units: 1 cm = 0.1 g/day (△ and ○), 0.5×10^{-4} gr.ion $[H^+]$/day (●).

and showed the same pattern of acidification and glucose decrease as the culture with thiamin alone, with the characteristic differences from the glucose-yeast culture, discussed already above. The Difco-aminoacid mixture as such and the combination with the vitamin factors has not yet been tried.

Figure 11 shows a titration curve of a culture solution (glucose-thiamin) in which *Omphalia flavida* has grown for $3\frac{1}{2}$ weeks (A), as compared with the picture for a fresh medium (B). There is a fairly constant difference of $\sim$ 14 ml 0.1n NaOH requirement per 100 ml solution. Interesting is the rather flat range between pH 3.8 and 5.2, indicating the possibility that the acids produced have their pK values somewhere in this region. All this will have to be persued further.

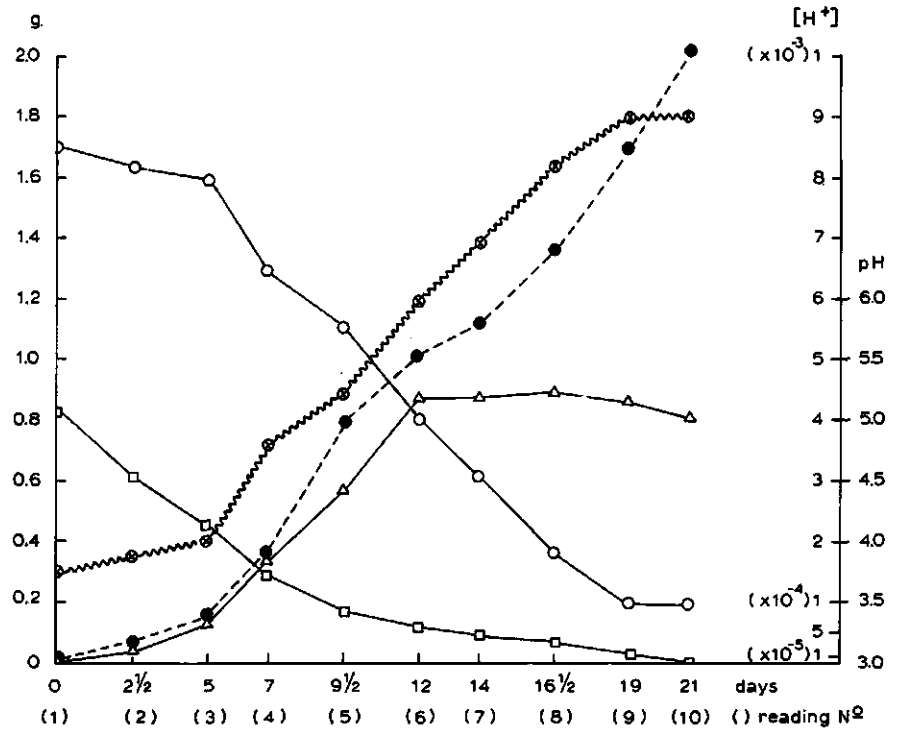


FIG. 7. Glucose-thiamin culture.
Dry weight of mycelium in 150 ml medium (△)] left
Glucose in 150 ml medium (○)] scale.
Glucose in 150 ml medium, inverse (⊗)
pH (□), [H+](●), right scales.

4. *Respiration in relation to mycelial ball size*

Comparative measurements of respiration (O_2-uptake) and light emission, and correlation with the growth phase of the culture required the development of a suitable mycelium dosage procedure and an estimation of the relation between the mycelium ball size and the rate of respiration. For this sake the inoculation material of a growth series (20 flasks of 750 ml each with 150 ml culture medium) was finely fragmented with the aid of a chemically sterilized mixer. After ca. 1 week of growth, the measurements were started. At successive harvest dates, or sometimes at the same harvest date, the mycelia balls present were sieved with the aid of 4 sieves with increasing mess-width, so that 4 size fractions were obtained (fig. 12).

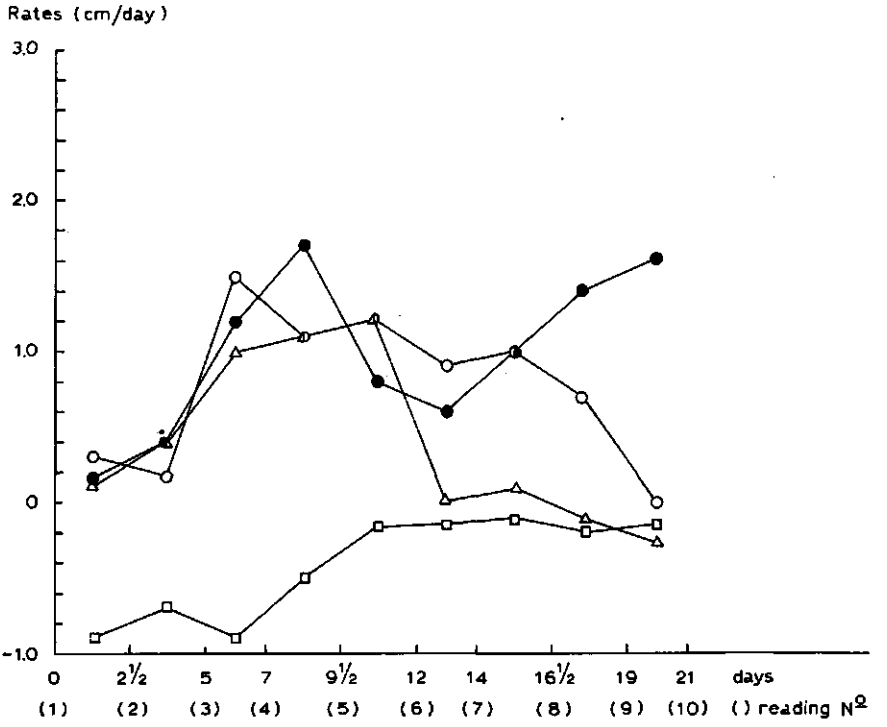
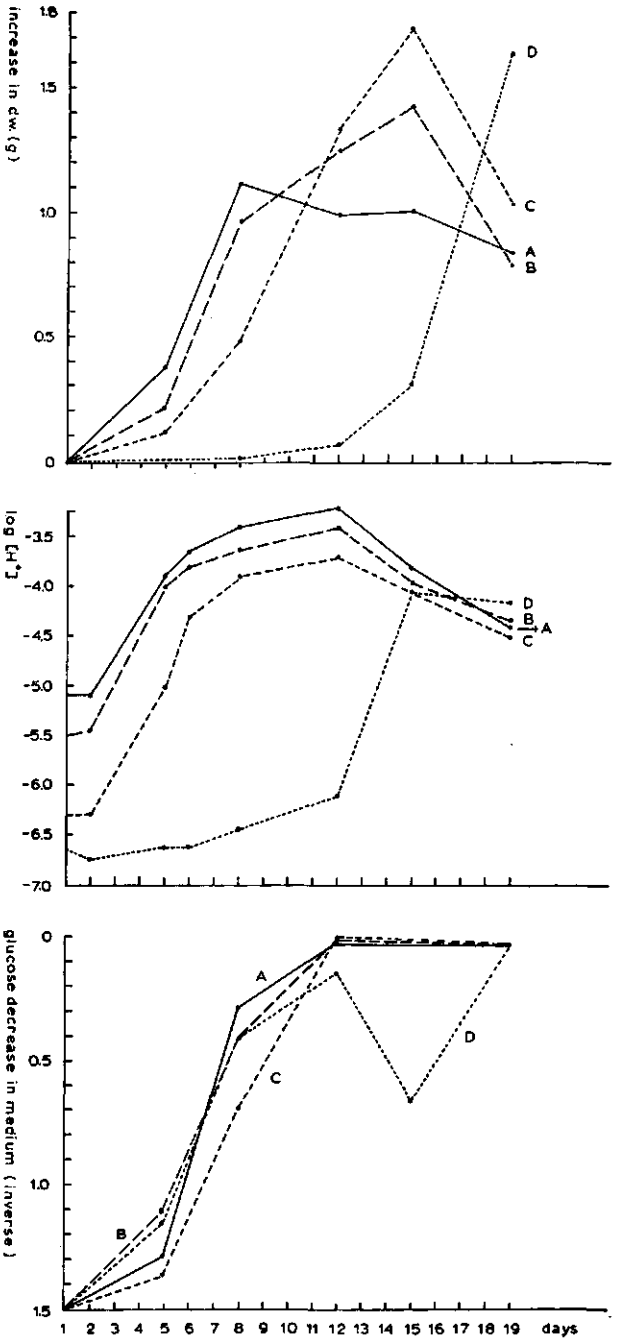


FIG. 8. Glucose-thiamin culture.
Rates (cm/day) of dry weight increase (△), glucose decrease in medium (○), change in pH (□), and change in H^+ (●).
Units: 1 cm = 0.1 g/day (△ and ○), 0.25 pH/day (□), 0.5 × 10^4 gr.ion [H^+]/day (●).

FIG. 9 (p. 13). Glucose-yeast cultures.
Effect of buffers of different pH on dry weight production, log [H^+] and glucose consumption.
Curves marked A: KH_2PO_4 1/2% (normal medium),
B: phosphate buffer pH 5.59,
C: phosphate buffer pH 6.47,
D: phosphate buffer pH 7.17.


increase in dw. (g)
1.8
1.5
1.0
0.5
0
D
C
A
B
log [H+]
-3.5
-4.0
-4.5
-5.0
-5.5
-6.0
-6.5
-7.0
D
B
A
C
glucose decrease in medium (inverse)
0
0.5
1.0
1.5
A
B
C
D
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 days

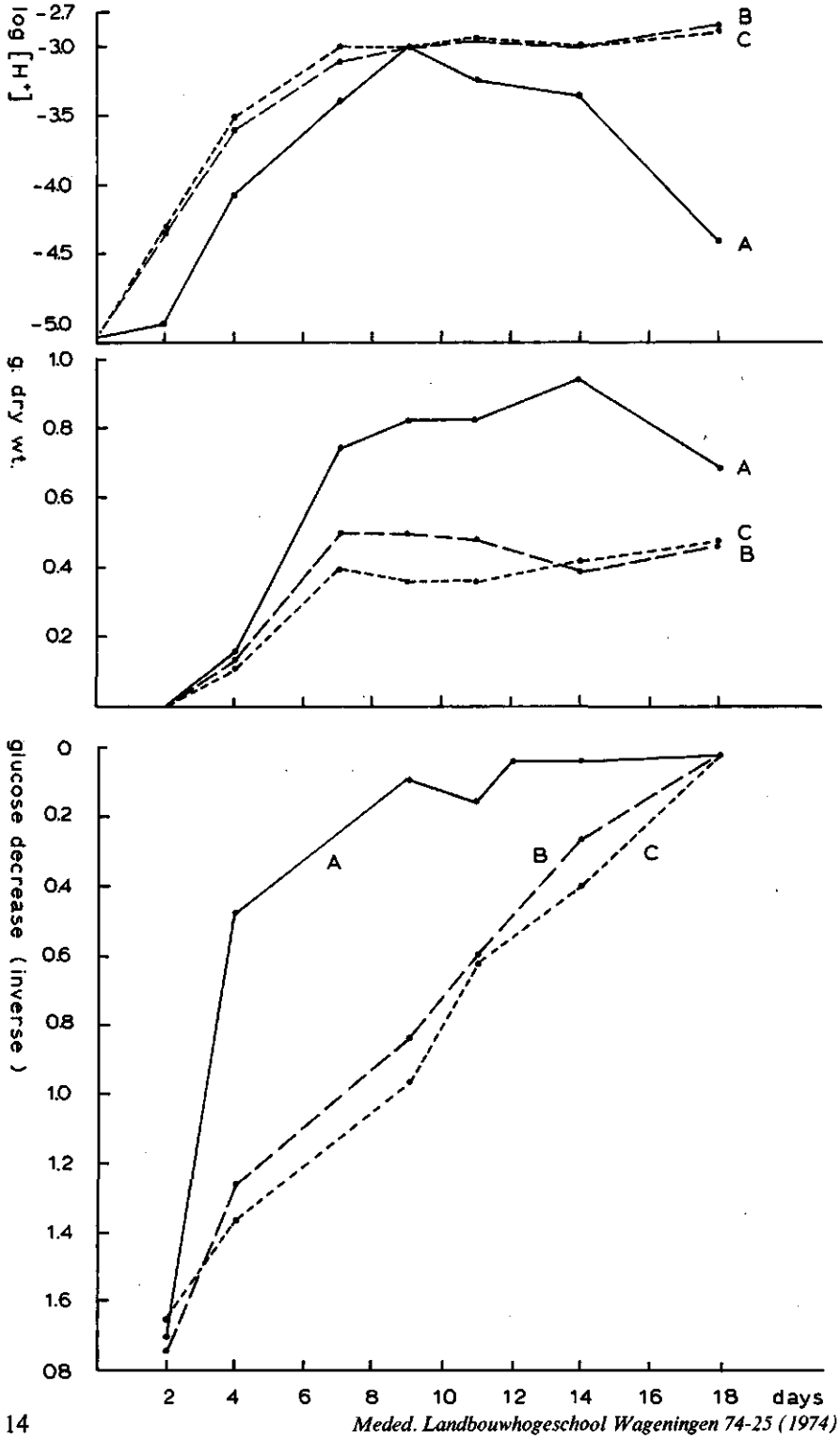

log [H+]
-2.7
-3.0
-3.5
-4.0
-4.5
-5.0
B
C
A
g. dry wt.
1.0
0.8
0.6
0.4
0.2
A
C
B
glucose decrease (inverse)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0.8
A
B
C
2
4
6
8
10
12
14
16
18
days

FIG. 10 (p. 14). Dry weight, sugar decrease (inverse) and log [H^+] for cultures on glucose yeast (Difco, 0.2%) (A), glucose thiamin (10^{-6} = 0.15 mg/150 ml) (C), and glucose vitamin mixture as indicated for Difco (pyridoxin 6.10^{-3} mg; biotin 3×10^{-4} mg; thiamin 9×10^{-4} mg; nicotinic acid 8.4×10^{-2} mg, riboflavin 6×10^{-3} mg, all in 150 ml) (B).

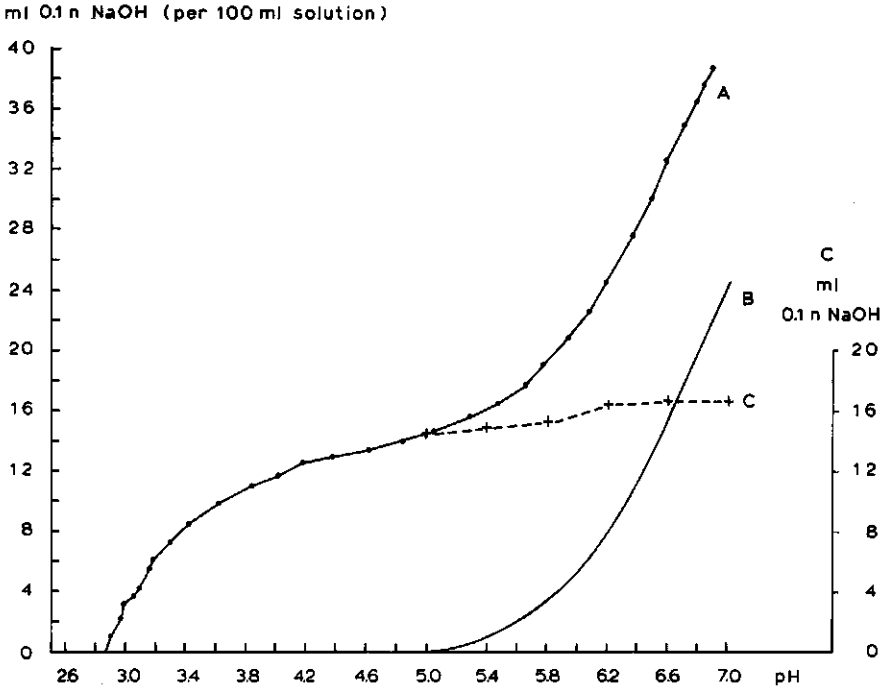


FIG. 11. Acidity difference (C) between a standard culture solution (glucose-thiamin) in which *Mycena citricolor* has grown for 3½ weeks (A) and a fresh medium (tentative curve, B, recently corroborated by actual titrations).

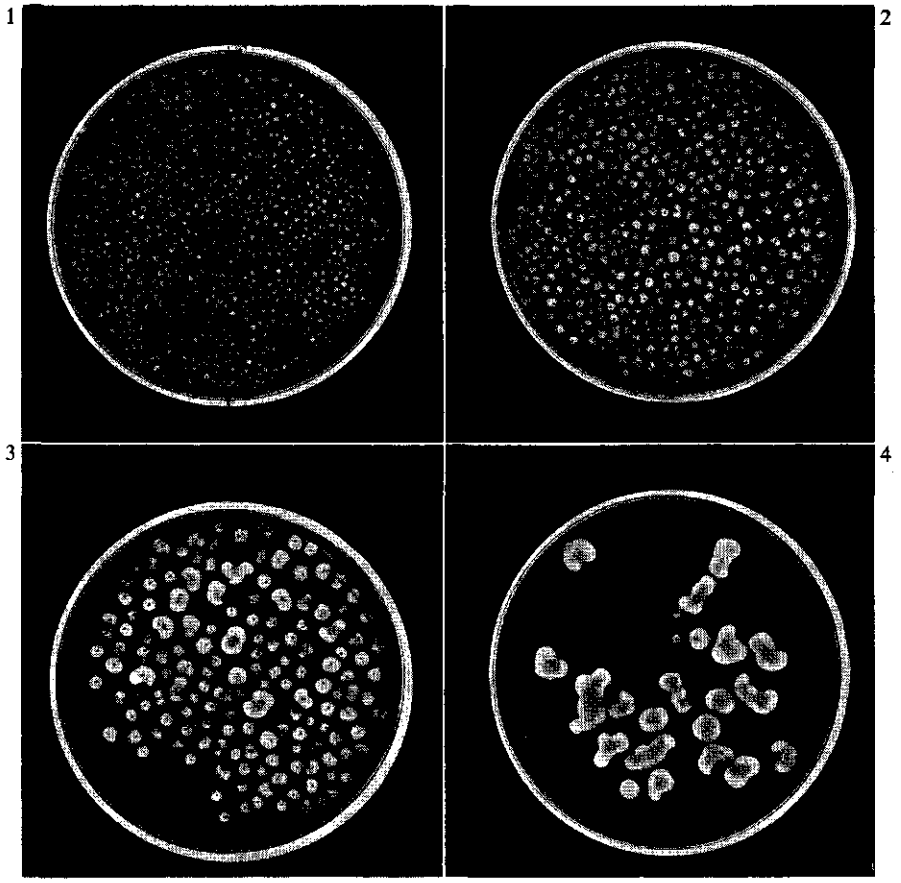


FIG. 12. *Omphalia flavida*, glucose-thiamin culture, 5 g fresh weight, age 18 days. 4 sieve fractions yielding different mycelium ball sizes, as used for comparative respiration measurements. No. 1: ca. 1260 balls, No. 2: ca. 400 balls, No. 3: 150 balls, No. 4: 23 balls, from 5 g fresh weight. Black dots in mycelial balls: counting marks.

Fig. 13 shows the number of balls per mg dry wt. (glucose-thiamin culture) in relation to ball size as expressed by r, r^2, or r^3 (standing for radius, surface and volume of individual balls). Notice the different abscissa-values.

Fig. 14 shows an average of 4 experiments (glucose-thiamin medium) relating rate of O_2-consumption (expressed in mm/mg dw/h) to total ball radius, total ball surface, total ball volume and number of spheres per mg dw. The respiration measurements were made with the WARBURG technique; 1 mm reading equals about 4 cmm O_2.* The results clearly show that, if 1 mg dw. of myce-

* Thus, 'normal-size' vessels, not the giant-apparatus, pictured in ref. (2), fig. 5, p. 261.

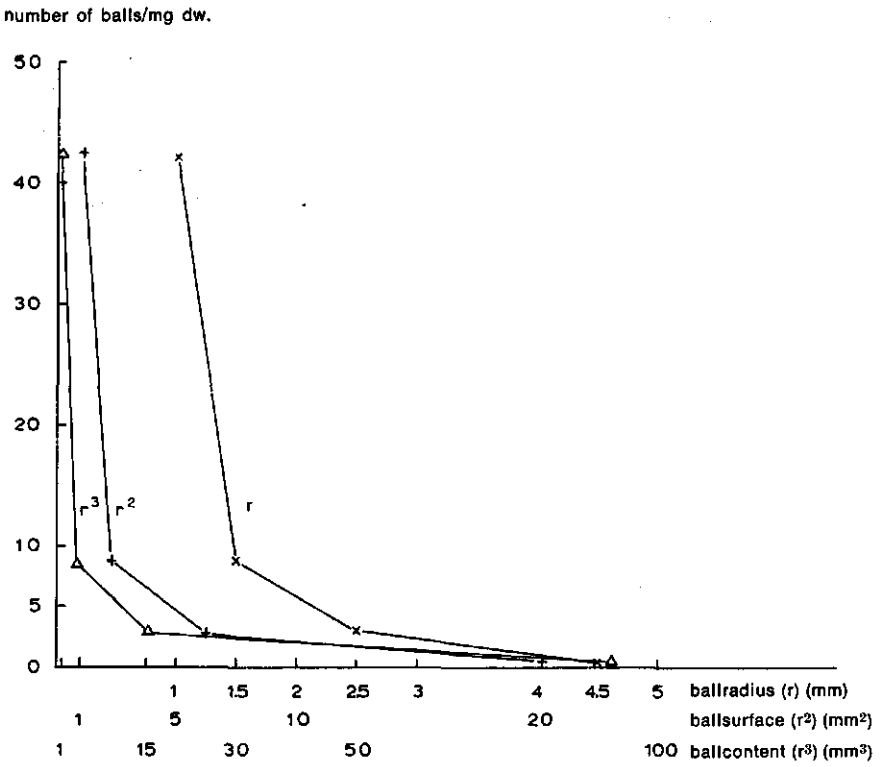


FIG. 13. Glucose-thiamin culture.
Number of mycelium balls/mg dw. against average ball radius (r, ×), ball surface (expressed as r^2, +), and ball volume (expressed as r^3, △). Different abscissae.

lium is divided up into more (and thus smaller) spheres, the respiration rate comes out higher. It should be remarked, that no sharp segregation is possible between the size effect and an age effect, anyhow always more than one ball size was derived from the same culture flask, sometimes all sizes, as in the example shown in fig. 12. It should further be remarked that it seems understandable – as a matter of fact this was the reason why these experiments were made – that size may affect respiration by the changed relation between surface and volume of the separate balls. The larger balls are very compact structures and it appears probable that their inner parts are increasingly liable to limitations of the rate of respiration by diffusion processes.

It should be noticed furthermore that the curve for respiration rate/mg dw. against total volume/mg dw. has a feature quite different from the others. Obviously, this is logical, since, if the specific weight were = 1, total volume would mean total weight which, in this case is 1 mg dw. as a calculation base for all cases. Ideally, thus, it might be expected that this curve should be represented by a vertical line from the highest point to the lowest i.e. from the smallest to

the largest balls. In fact, there appears to be a rather considerable spreading; the middles of the connecting lines show a more or less vertical, straight line, pointing to a slightly larger volume (slightly lower specific wt.) in the larger ball sizes. It is, however, uncertain so far whether this has any real significance; it might point to the possibility that the larger spheres contain some light materials, e.g. lipids in their interior which, in view of presumable respiration and ageing conditions, cannot be excluded.

It may be asked whether extrapolation of the curves of figure 14 to zero is justified. Considering the relation of respiration to, e.g., number and total r (per mg dw.), it lays at hand to consider this extrapolation justified. The zero value indicates that respiration goes to zero if number, total r, and total surface *per mg dw.* become infinitely small, in other words when the individual balls grow infinitely large.

In order to explore this somewhat further, in figure 15 the rate of O_2-uptake (mm per mg dw/h) has been related to r, r^2 and r^3 of individual balls. It shows how the rate of respiration of 1 mg dw. depends on individual ball size, viz., the fineness of partition.

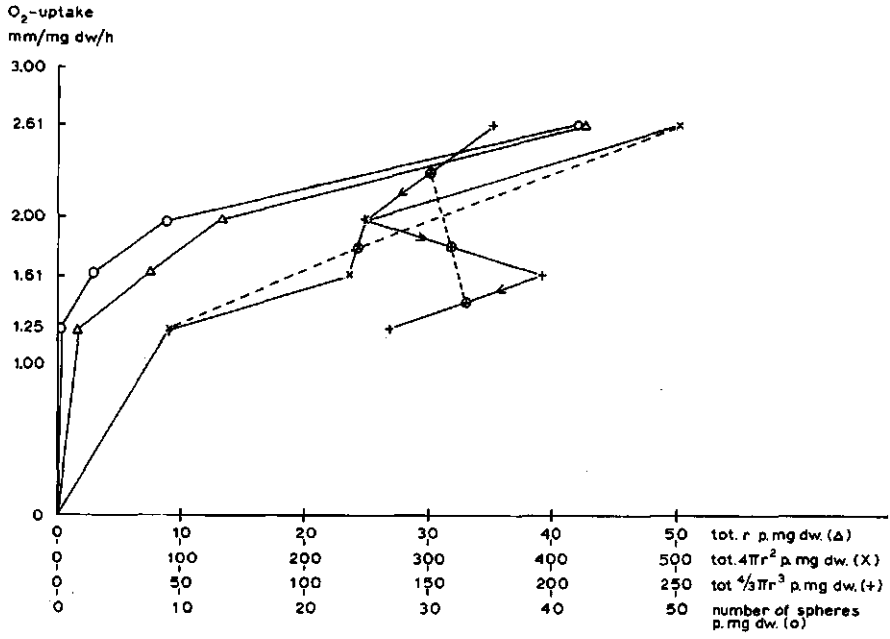


FIG. 14. Glucose-thiamin culture.
O_2-uptake in mm/mg dw./h in relation to ball size.
○: against number of balls p.mg dw.
△: against Σ r (total r) p.mg dw.
×: against $\Sigma 4\pi r^2$ (total ball surface) p.mg dw.
+: against $\Sigma 4/3\pi r^3$ (total ball volume) p.mg dw. (⊗ averages of 2 successive readings).
1 mm reading = ~ 4 cmm O_2

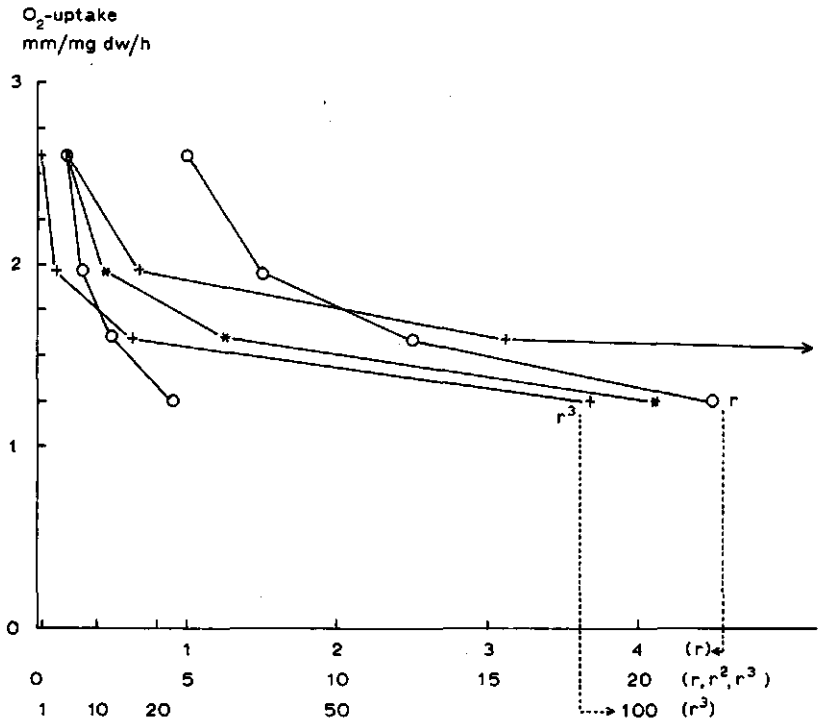


FIG. 15. Glucose-thiamin culture.
O_2-uptake in mm/mg dw./h. in relation to *average individual ball size* (expressed in r (○), r^2 (*) or r^3 (+) at the same abscissa. Moreover: r at 5× abscissa, r^3 at 1/5 of common abscissa. Ordinate: 1 mm = ~4 cmm O_2.

In figure 16 the curve for r^2 from figure 10 has been replotted, putting size (r^2) on a logarithmic scale, and an (admittedly rather arbitrary) extrapolation has been attempted to a near-to-zero value for the respiration rate. From the values obtained, the corresponding r-values have been plotted in figure 17. The procedure suggests that zero-respiration/mg dw. is reached around $r = 10^3$ mm (individual ball size). It should be remarked that the procedure is tentative and does not claim any appreciable accuracy; it may be added that extrapolation from respiration rates against individual r-values, instead of r^2-values yields figures for r at zero respiration/mg dw. around 10^2 mm.

It does not appear improbable that imaginary mycelium balls with $10^2 < r < 10^3$ mm should show a respiration rate next to zero per mg dw/h; considering that the dry wt. of such balls may be in the order of 5 kg.

5. *Comparative studies on growth, respiration rate and luminescence intensity*

The experiments on the effects of ball size were made as an introduction to kinetic series aiming to compare, during the growth of cultures, dw. develop-

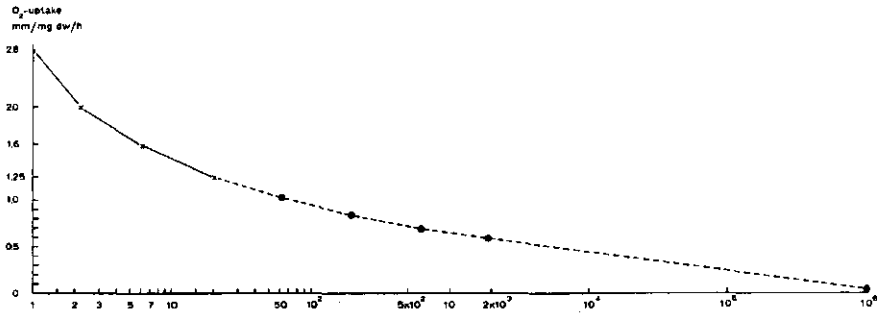


FIG. 16. Glucose-thiamin culture.
O_2-uptake (mm/mg dw./h) in relation to r^2 of individual balls (semi-log scale), extrapolated to O_2-uptake $\sim$ 0 mm/mg dw./h ($r^2 \sim 10^6$ mm^2).

ment, rate of respiration and light intensity, both for glucose-yeast and glucose-thiamin cultures. Series of 20 or more flasks for each medium were simultaneously inoculated with finely divided material, grown 7 days before in glucose-yeast medium from blendered mycelium.

Determinations were made every 2 or 3 days and accumulated dw., rate of respiration, and light intensity recorded. The material consisted mainly of sieves 1 and 2 (fig. 12) only. Rate of respiration was calculated, as before, in mm O_2/

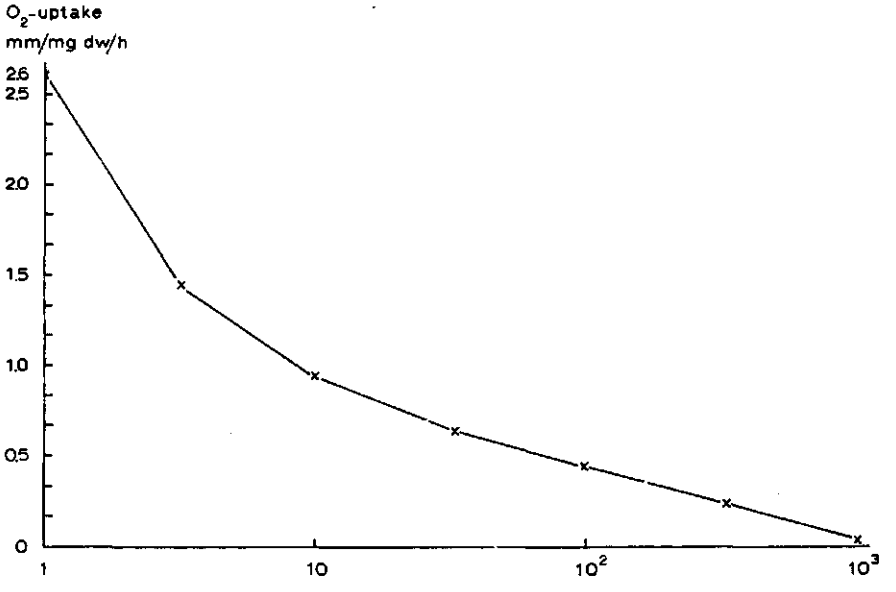


FIG. 17. Glucose-thiamin culture.
O_2-uptake (mm/mg dw./h) against r of individual balls, from extrapolation of same against r^2 until O_2-uptake/mg dw. near zero ($r \sim 10^3$ mm).

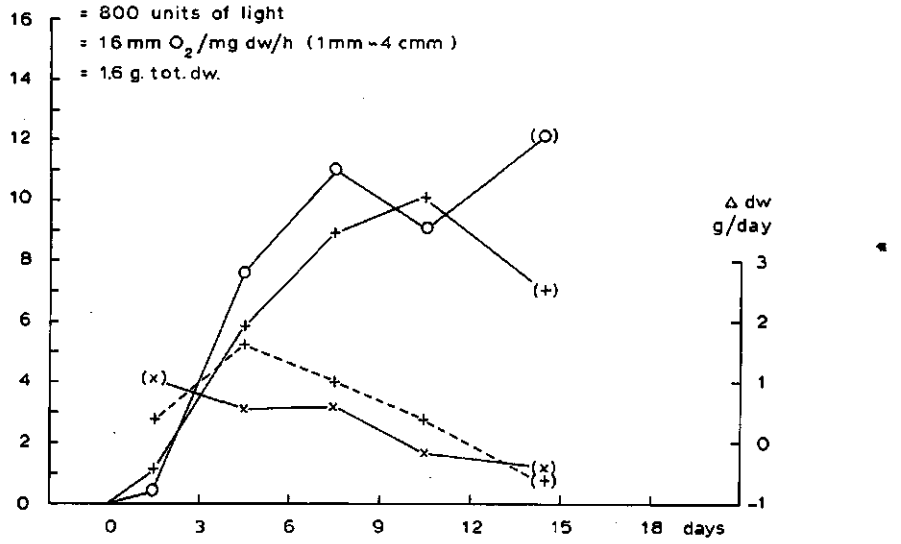


FIG. 18. Glucose-yeast culture (150 ml).
Comparative measurement of accumulated dry wt. (g, +), O_2-uptake (mm/mg dw./hr, ×), light intensity (arbitr. units, ○) and △dw. (g/day, ---×---).
Units: Left scale: 1 scale unit = 50 arb. units of light; = 1 mm O_2/mg dw./hr (= ~ 4 cmm O_2); = 0.1 g tot. dw. Right scale: △dw in g/day. Abscissa: Age of culture in days, av. of 2 exp's, points between ( ): Exper. II only. Ball size: fraction No. 1 + 2

mg dw./h. Light intensity was determined in arbitrary units with an apparatus consisting of a large surface photomultiplier, connected with an IHU-Photometer power supply. The combination was provided by Dr. C. J. P. SPRUIT from our laboratory, and was suitable (with different ranges of sensitivity) for measuring luminescence of 150 cc cultures or for measuring in WARBURG vessels. Figures 18 and 19 each show average results of 2 similar experiments, for glucose-yeast and glucose-thiamin cultures respectively.

The dw accumulation in fig. 18 shows the features previously discussed, fig. 19 shows that dw accumulation in the glucose-thiamin medium is much slower and reached a much lower level. Light production is very much higher in the glucose-yeast culture than in the medium with thiamin. Earlier qualitative evidence that light emission often persists or even increases after, sometimes long after, the culture has reached maximum dry weight, appears supported in the glucose-yeast medium. Respiration rate, remarkably, is less lower in the thiamin culture as compared with the yeast containing medium than any of the other features discussed. Since respiration and light are expressed as rates or intensities, we have also calculated the rate of dw. accumulation per day. Especially in the glucose-yeast medium it is evident that the rate of respiration gradually slows down slightly during the culture period, more or less paralleling a similar decrease in the rate of dw. accumulation.

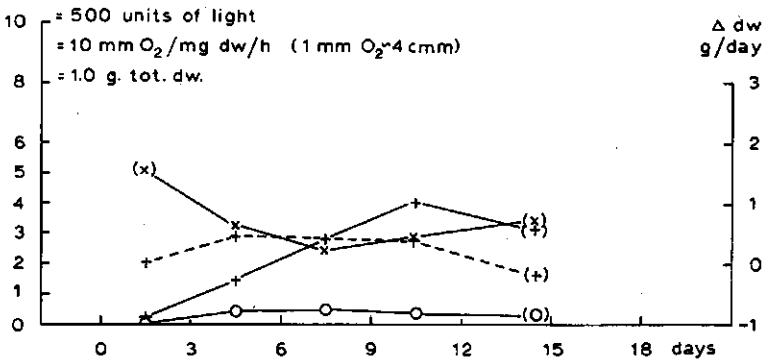


FIG. 19. Glucose thiamin culture (150 ml).
Comparative measurement of accumulated dry wt. (g, +), O_2-uptake (mm/mg dw./hr, ×), light intensity (arbitr. units, ○), and △dw. (g/day, ---+---).
Units: Left scale: 1 scale unit = 50 arb. units of light; = 1 mm O_2/mg dw./hr (= ~ 4 cmm O_2); = 0.1 g tot. dw. Right scale: △dw. in g/day. Abscissa: Age of culture in days, av. of 2 exp's, points between ( ): exper. II only. Ball size: fractions No. 1 and 2.

It should be observed that the rate of dw. accumulation smoothly decreases over the greater part of the curve and proceeds without specific irregularities from positive to negative values in the later parts of the growth period. The latter features are somewhat less clear in the case of the thiamin cultures owing to the lower values of most of the rates studied.

It should still be remarked that averages for both series were taken over periods of 3 days. The last 3-days period was available for one of the two experiments only.

The striking difference between the intensity of luminescence in the glucose yeast-medium and in the glucose-thiamin cultures suggests that the yeast-addition provides some factor specifically favourable for luminescence. It may be worth while to look into this in more detail.

6. *Caloric data and efficiency of energy conversion during growth*

Finally we will discuss some preliminary data on caloric values and the efficiency of energy conversion in the process of producing fungus material from glucose.

Table 1 shows some representative determinations which were possible through the courtesy of Dr. A. J. H. VAN ES and some others.

There is a fairly high ash content (~ 25% of rough dw.), probably owing to retention of salt from the culture medium inside the mycelium balls. Taking this into account, the caloric value of the mycelium per g organic material was 4001 cal in the older culture, of exp. A, and 3479 cal in the younger culture, of exp. B, not differing very much from that of carbohydrate. One might have expected slightly higher values. The overall efficiency of glucose conversion into mycelium (cal/cal) was very near to 40% in both experiments.

Table 1. Caloric data concerning *Mycena citricolor* cultures.

Exp. A. 22.3.'72–17.4.'72	Exp. B. 7.4.'72–17.4.'72
5-l Flask, glucose-yeast medium	Same as A.
Dry wt mycelium 7.47 g	9.47 g
Total consumed glucose 14.520 g	14.609 g
Cal/g *2865*	*2355*
Carbon content 28.29%	23.29%
Ash content 24.1%	26.9%
Moisture content 4.2%	5.4%
Organic matter 100 — 24.1 — 4.2 = *71.6%*	*67.7%*
Organic dry wt. 71.6/100 × 7.47 = 5.349 g	67.7/100 × 9.47 = 6.411 g
Carbon content in organ. matter 39.5%	34.4%
Cal/g organ. matter 100/71.6 × 2865 = *4001*	100/67.7 × 2355 = *3479*
Cal in mycelium 7.47 × 2865 = 21402	9.47 × 2355 = 22302
Cal in glucose: 14.520 × 3750 = 54450	14.609 × 3750 = 54782
Efficiency (cal/cal) 21402/54450 × 100 = *39.31%*	22302/54782 × 100 = *40.71%*

Some preliminary experiments in which this efficiency was computed, after irregular early data, showed a tendency to boil down to very similar figures in the later experiments. Earlier (2), we had computed the efficiency of conversion of bread extract into fungus material in large full-grown cultures. On the average, 56.6 g dw. of bread introduced produced 21.0 g dw. of mycelium, yielding an efficiency value of 0.39, an almost exact coincidence with the later experiments on glucose media.

This conversion factor is lower than the one usually found and suggested for bacteria and fungi (~0.6).

Figures 3 and 7 suggest differences in the efficiency of energy conversion during the course of development of a culture. They especially suggest high yields during the period of vigorous growth, when the curves for dw. increase and sugar consumption run closely parallel and near to each other.

Data in Table 2 have been obtained using the determinations from Table 1 in connection with the data of figs. 3 and 7. They indeed show high yields (up to ~90%) in the period of vigorous growth, strongly decreasing thereafter. The data are represented graphically in fig. 20. The general course for glucose-yeast and glucose-thiamin cultures is more or less the same, notwithstanding distinctly lower values in the thiamin cultures, as observed before. The overall efficiency during the entire culture period comes down to figures, very similar to those reported above and earlier.

A thorough evaluation of the rates of dw. accumulation, sugar consumption, respiration and acidification has not yet been carried through and seems worthwhile.

7. *Future problems*

It should be added that the physiological features discussed so far were all obtained using submerged growth in shake cultures. Essentially, they are confined to rather early stages of mycelium growth. Connection with surface growth

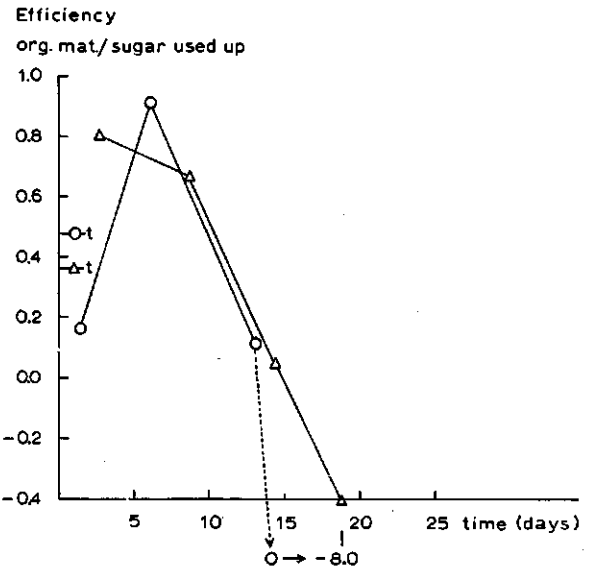


FIG. 20. Course of the efficiency of energy conversion during growth in glucose-yeast (○) and in glucose-thiamin (△) cultures; t = total (average over entire period).

which, especially in bread extract cultures, occurred after the space inside the liquid medium was fully occupied by the fungus, have not yet been made. From experience obtained earlier with surface growth of *Phycomyces* (3), it may be expected that rate of dw. accumulation and rate of respiration per unit dw. will continually decrease, down to very low values. Incidentally, it has been observed, especially in bread cultures, that patches of mycelium that, during shaking, become settled on the wall of the flask above the normal amplitude of the liquid surface, show a much brighter luminescence than the submerged parts. This may indicate that the better separation of aerial mycelium threads, and thus the better availability of oxygen, is responsible herefore. As a first further step, the dependency of the luminescence of submerged cultures to oxygen tension will have to be looked into.

Some observations, made very long ago, point to the possibility that specific poisons, e.g. cyanide, may affect luminescence much less than, e.g., oxygen consumption (6). Also this has not yet been followed up further.

It has once been our aim to provide the chemist, interested in isolating luminescent material and enzymes with a prescription for obtaining cell-free luminous systems from submerged cultures. However, any progress in this direction has not yet been made.

Finally, the low intensity of fungus luminescence, and more or less of all bioluminescence phenomena, is worth discussion. Bioluminescence, in an organism proves to be an improbable phenomenon, which does not seem surprising, if one considers that, out of energy packages of the order of 10 kcal/

Table 2. Efficiency of energy conversion in cultures during growth.

Glucose-yeast culture (fig. 3)						Glucose-thiamin culture (fig. 7)				
Time (d)	dw. (g/150 ml)		Org. mat. (dw 0.70)	Sugar used up (g/150 ml)		Efficiency (cal/cal)	Time (d)	dw. (g/150 ml)	Org. mat. (dw 0.70)	Sugar used up (g/150 ml)	Efficiency (cal/cal)
0 – 2½	0.04		0.03	0.18	0.18	0.17	0 – 2½	0.12	0.08	0.10	0.80
2½– 5	0.31			0.20			2½– 5				
5 – 7	0.45	1.38	0.88	0.40	0.97	0.91	5 – 7				
7 – 9½	0.52			0.37			7 – 9½	0.75	0.53	0.80	0.66
9½–12	0.03			0.17			9½–12				
12 –14	0.00	0.08	0.06	0.30	0.50	0.12	12 –14	0.03	0.02	0.44	0.05
14 –16½	0.05			0.03			14 –16½				
16½–19	–0.15	–0.23	–0.16	0.01	0.02	–8.0	16½–19	–0.10	–0.07	0.17	–0.41
19 –21	–0.08			0.01			19 –21				
	Total		0.81	1.67					0.56	1.51	
	Cal/gr		3740	3750					3740	3750	
	Cal tot.		3030	6300		0.48			2100	5650	0.37

Mol (ATP and similars) a molecule has to be built up capable of releasing at once an energy jump of the order of ~ 50 kcal/Mol.

8. *Summary*

This paper gives the results of some preliminary physiological experiments on the luminescence of the fungus *Omphalia flavida* (= *Mycena citricolor*) as a continuation of earlier work (ref. 1, 2). The following types of experiments were made:

1. Growth in submerged state in shake cultures in liquid medium with a completely determined C-source. This was necessary for kinetic studies and was possible by addition of a small amount of a vitamin mixture (purified yeast extract) or of thiamin; the latter provided about half the growth of the former (see e.g., fig. 2).

2. Kinetic studies on growth, glucose consumption and acidification of the medium in cultures with yeast extract or thiamin addition; the mentioned phenomena are strongly related, see e.g. figs. 3 and 7.

3. Relation between the rate of respiration per unit dry wt. and the size of the mycelium balls as developed in the shake cultures. The main results of these experiments are in figs. 14 and 15.

4. Kinetic studies on the rate of respiration in relation to dry wt. accumulation, sugar uptake, light emission and acidity development. The main results of these experiments are in figs. 18 and 19.

5. Determination of the caloric value of the organic mycelial material, and computation of the efficiency of the energy consuming process. These data are contained in Tables 1 and 2, and in fig. 20.

9. *Acknowledgement*

The start of this work was possible by a grant from the organization S.O.N. (Chemical Research in the Netherlands) which enabled to appoint Miss I. RIPKE (later Mrs. I. RIJNIERSCE-RIPKE) as a technical assistent. The author is indebted to Mrs. RIJNIERSCE for skill, intelligence and devotion shown in performing and discussing the experiments; to Dr. A. J. H. VAN ES, Dr. C. J. P. SPRUIT, and Drs. G. H. M. KRONENBERG for scientific support, and to Miss M. E. VAN DEN NOORT for careful drawing of the graphs, and valuable help in the correction of the proofs.

10. *References*

1. WASSINK, E. C. Rec. trav. bot. néerl. **41**, 150–212 (1948).
2. WASSINK, E. C. and S. KUWABARA. In: Bioluminescence in Progress (F. H. JOHNSON & Y. HANEDA eds.), Princeton (U.S.A.), 1966 pp. 247–264.
3. WASSINK, E. C. Rec. trav. bot. néerl. **31**, 584–690 (1934).
4. Cf. ref. 3, p. 603.
5. SCHOPFER, W. H. Plants and Vitamins, Waltham, Mass, U.S.A., 1949 pp. 1–293, especially pp. 101 ff.
6. WASSINK, E. C. and A. VAN DER BURG. Unpublished observations.
7. KUWABARA, S. and E. C. WASSINK. In: Bioluminescence in Progress (F. H. JOHNSON & Y. HANEDA eds.), Princeton (U.S.A.), 1966 pp. 233–245.