Nonthermal Effects of Millimeter Microwaves on Yeast Growth

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Weak microwave irradiation of aqueous yeast cultures was found to affect their growth rate in a frequency-selective manner. Depending on frequency (near 42 GHz), both increases and decreases of the growth rate were observed. The resonance bandwidths are of the order of 0.01 GHz. Simple thermal effects can be excluded. These findings support theoretical predictions of coherent molecular oscillations activating metabolic processes.

Introduction

From theoretical considerations Fröhlich has suggested that strong oscillations, *e. g.* in macromolecules, might play a basic role in active biological systems [1]. The frequencies of these oscillations were estimated to be of the order of 100 or 1000 GHz. The general idea is that an attractive force might exist between two partners of an enzymatic reaction, for example, if they are both excited to an oscillation at the same frequency. The excitation could come from the release of metabolic energy thus switching on the enzymatic activity.

Recent experiments [2–5] seem to support Fröhlich's conjecture since they show a biological response to weak irradiation with electromagnetic waves near 100 GHz, together with a resonance-like frequency dependence. These effects can possibly be understood by assuming that the externally applied radiation drives the oscillation and thus the activity of a relevant enzymatic molecule which happens to be on resonance.

It is the purpose of this paper to describe in detail our experimental procedure and results on the influence of coherent microwave irradiation on the growth of yeast [5]. Frequencies were chosen in a range near 42 GHz, as in a yeast experiment mentioned by Devyatkov [3]. Our main concern was to achieve a high degree of reproducibility. In contrast to refs. [2–4] we did not measure just a growth factor for each growth experiment, since we observed a nonexponential growth behaviour for the first few hours of each growth experiment. Instead, we continuously monitored the cell density optically and thus were able to measure directly the exponential growth rate.

To avoid thermal effects of irradiation we decided not to use cultures growing on Agar surfaces. For such samples the thermal coupling between cells and substrate can be poor and might lead to a non-negligible heating. Then a small temperature rise might enhance the growth rate while overheating beyond about 37 °C is known to decrease it. Instead we chose to use a yeast cell culture in stirred aqueous suspension, as this assures an efficient thermal coupling between the cells and their surrounding. The temperature of the suspension was continuously monitored.

Finally a measurement of the irradiation intensity is difficult because of strong interference effects. To have a calibrated and continuous monitoring of the absorbed microwave power we thermally isolated the sample cell and used it as a calorimeter: The temperature of both the sample and its surroundings were measured, the difference being proportional to the absorbed power.

Material

We used a diploid, homozygot and isogene wild type strain of *Saccharomyces cerevisiae* (type 211 [6]). Reproduction of these cells occurs vegetatively by mitosis only. Cells grew on agar plates (20 g/l Bacto agar, 20 g/l D-glucose monohydrate and 5 g/l Bacto yeast extract) for three days at 30 °C. These plates were then stored at 4 °C. Cells for liquid suspension were taken from these plates after 10 to 16 days.

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The liquid medium contained 20 g/l D-glucose monohydrate and 5 g/l Bacto yeast extract. It exhibited pH = 6.1. Yeast cells were suspended with a starting concentration of 2.3 to 4.5×10^5 cells/cm^3. This defined time zero for our growth experiments. 100 cm^3 of suspension was kept at 32 ± 1 °C for about one hour, then a sample was taken into the measuring cuvette.

Growth behaviour

Yeast cells stored at 4 °C as described above are known to gradually approach stationary synchronization in the so called G1 phase of the cell cycle. There they exhibited a relatively broad volume distribution as shown in Fig. 1. This result was measured with a particle volume analyser (Coulter Electronics). Different environments on the agar surface were thought to be responsible for the widely varying volumes.

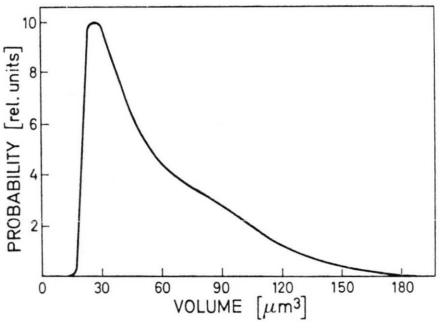

Fig. 1. Volume distribution of resting yeast cells.

If the cells were then suspended in growth medium at 32 °C, as described above, a relatively long time of a few hours was needed until the first cell division occurred. This time varied strongly with temperature, but also with the initial volume of a yeast cell. After the first division the cell cycle time, *i. e.* the reduplication period (about 75 min), was found equal for all cells regardless of history [7]. This behaviour is known from observations of individual single cells on agar plates. Some results of Nüsse are reproduced in Figs. 2 and 3. We measured the time needed for the first division in our sample by microscopic observation. A broad double-peaked distribution resulted (Fig. 4) which reflects the initial volume distribution of Fig. 1 in overall agreement with Fig. 3. Altogether, we could therefore expect that the initial synchronization was nearly completely lost at the time of the first cell division.

Limits of growth were imposed on our aqueous culture by changes in the growth medium. The amount of Bacto yeast extract used was chosen to

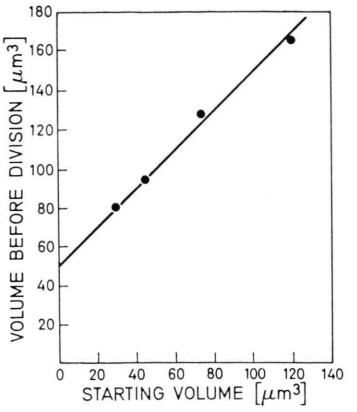

Fig. 2. Correlation of yeast cell volume in the stationary G1 phase ("starting") with that in the phase just before the first division occurs (after Nüsse [8]).

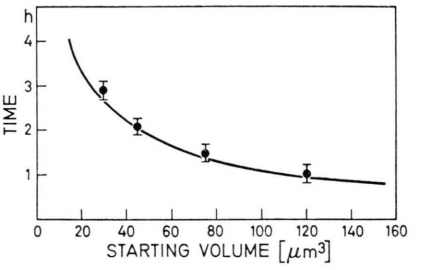

Fig. 3. Dependence of the time needed until the first division occurs on the cell volume in the stationary G1 phase (after Nüsse [8]).

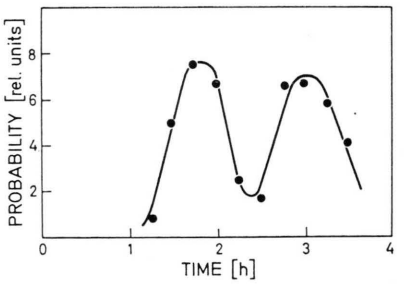

Fig. 4. Distribution of the time needed until the first division.

last for about six or seven generations. pH decreased from an initial value of 6.1 to about 5.0 after ten hours. Our cultures thus remained within the optimal, pH-independent growth range [9] for all our measurements. The oxygen intake was calculated to be 10^{-6} mol corresponding to a gas volume of 0.02 cm^3 per cm^3 of our aqueous culture growing for 10 hours. With the geometry used in our experiments oxygen depletion was therefore not likely to be reached.

Measurement of growth

The growth medium appeared yellow-brownish, optically translucent, while the yeast cells strongly scattered visible light. Thus the cells added significant cloudiness from the beginning of growth.

This light scattering was used to measure the growing cell concentration, by observation of the extinction of a transmitted light beam of 525 nm wavelength. This method offered a good measurement of the number density of cells as long as the volume distribution of the cells remained constant. But even during the initial hours of our growth experiments, where the number density was constant, the growth of the cells' volume (Fig. 2) was reflected in an increasing extinction. Actual recordings of extinction vs. time were taken with a Beckman Acta C III double-beam photometer. Standard glass cuvettes with $1 \times 1\ \mathrm{cm}^2$ internal cross section were used. The cross section of the measuring beams was 9 mm in heigth and 5 mm in width. The reference cuvette was filled with plain growth medium. The sample cuvette contained the growing yeast culture. Extinction E due to the presence of yeast cells was then defined by $E = -\log(T_S/T_R)$ where T_S and T_R were the transmissions of the sample and reference cuvette, respectively.

The sample cuvette was equipped with a special insert for mechanically stirring the suspension and for adducting the microwaves. A scale drawing is given in Fig. 5. At the beginning of each growth experiment, cell suspension was filled up to a height of 25 mm. Stirring caused a strong motion of the liquid, and some foam developed on the surface. After 11 hours about 20% of the liquid volume was lost, probably by liquid transport through the small bore provided for the moving stirrer shaft.

A typical recorder trace of the yeast extinction E vs. time is shown in Fig. 6. This is replotted on a logarithmic scale in Fig. 7. In general thus the extinction increased very slowly at the beginning but later asymptotically reached an exponential rise. In the neighbourhood of $E \cong 0.9$ a gradual slowing down of the growth was usually observed, probably caused by depletion of Bacto yeast extract in the medium.

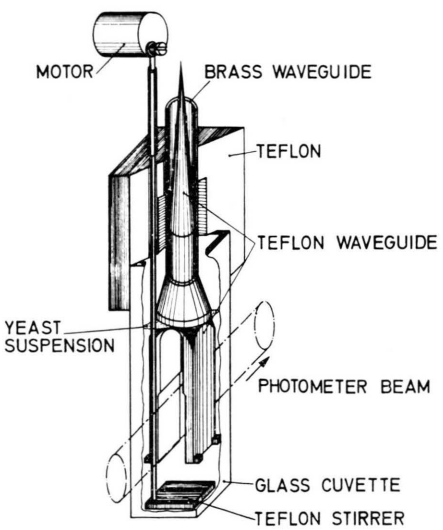

Fig. 5. Outline of photometer cuvette containing the growing yeast culture. The stirrer moves 7 mm up and down at a frequency of 2 Hz. Microwaves are aducted via the hollow metal waveguide and penetrate through the teflon antenna into the yeast suspension. The exponential power penetration depth into water is 0.2 mm at 42 GHz.

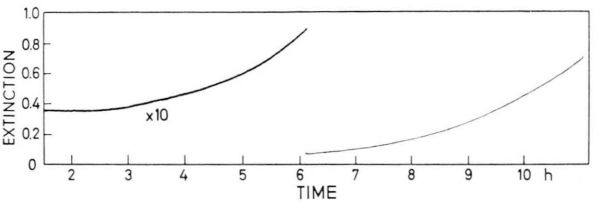

Fig. 6. Recorder trace of photometer extinction *vs* time.

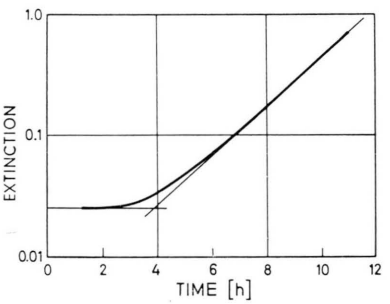

Fig. 7. Extinction growth curve of Fig. 6 replotted on a logarithmic scale.

Independent of this measurement, we had a second rather simple photometer at our disposal (Lange Model J Universal-Colorimeter). This we used for controlling the growth behaviour of the remainder (approx. 97 cm^3) of the suspension from which the sample cuvette had been filled. This control was not used as a reference in our experiments, but merely served to indicate a disturbed condition like infection or chemical poisoning.

Temperature controls

Temperatures were continuously recorded at three locations in all experiments. These served the twofold purpose of (i) finding he sample's temperature

T during the period of each recording, and (ii) finding the microwave power L absorbed by the sample during the same period.

(i) The temperature in the sample could not be measured with a submerged thermocouple during a run because of anticipated interference with microwave radiation. Therefore, we chose to have three miniature precision thermocouples to measure the temperature at nearby points; the rationale being that the microwave absorption represented a heat source of $10-50$ mW in the sample so that a steady-state temperature gradient formed between sample and its surrounding. For the small temperature differences involved, all thermal equilibration processes could be assumed to be linear, *i.e.*, sensing of the temperature difference τ between suitable points outside the cell provided a measure of the temperature difference ΔT between sample and background (T_b) according to $\Delta T = T - T_B = g \cdot \tau$, where the proportionality factor g could be found by calibration. We measured T_b at the wall of the compartment housing the measuring cuvette, and the two temperatures defining τ at the bottom of the measuring cuvette (T_1, spring contact) and at a suitable point on the structure holding the cuvette (T_2). The value of g was established by observing T_1, T_2 and T_b as well as T, the latter with an auxiliary thermocouple submerged in the sample, during the cooling process of water at 40 °C filled in the cuvette. During this calibration procedure the microwave guide stayed inserted and the stirrer stayed active but no microwave power was applied. From this we found $g = 2.0 \pm 0.2$ and 150 seconds as the characteristic time for decay of ΔT to 1/2 its value.

Altogether we thus continuously recorded ΔT and T with an accuracy of ± 0.1 °C and ± 0.2 °C, respectively. The variations of ΔT during any of the growth experiments never exceeded ± 0.1 °C. T_B, however, and thus T did vary by up to ± 0.5 °C due to slow temperature cycles in the laboratory for any growth experiment. The mean value of T_B and T was in the range 30.5 to 34.0 °C for all experiments.

(ii) The steady state heating effect of the microwaves ΔT was continuously measured by the above method. [In an independent calibration experiment ΔT was directly measured by submerging thin encapsulated liquid crystal foil (Edmund Scientific) into the sample cuvette, giving the same results. This thermometer exhibeted a fulspectrum color change in a range of 3 °C.] In this way we were assured of ΔT being 0.2 to 0.7 °C for all of our irradiation experiments. Linking this to the absorbed microwave power was possible by the mentioned cooling-down experiment. There we established the rate of decrease of ΔT to be 0.0046 sec^{-1}. Taking the heat capacity of 2.5 cm^3 of water to be 10.5 J/°C we found $L = 10.5$ J/°C $\times$ 0.0046 $sec^{-1} = 48$ mW necessary to sustain a sample over-temperature of $\Delta T = 1$ °C. This power agreed roughly with the maximum microwave power as specified by the tube supplier, 45 to 55 mW around 42 GHz.

While we were certain that ΔT measured the net power input in the cuvette we could not rule out some heat sink contribution arising from evaporation cooling of the sample. Observations showed that the sample volume decreased by 0.5 cm^3 during a ten hour experiment, from which a maximum cooling power of 35 mW could be calculated. However, the actual cooling effect on the liquid could be assumed much smaller, since a tight teflon lid prevented direct evaporation from the surface, and the liquid loss seemed to occur mainly by liquid transport through the small bore provided for the moving stirrer shaft. We were not able to detect such a cooling effect with a thermometer resolution of 0.1 °C, and therefore, disregarded this effect.

Microwave system

A submerged dielectric antenna was used to irradiate the yeast suspension over a surface of about 10 cm^2, as shown in Fig. 5. The microwaves entered this antenna from a round brass waveguide ending just above the liquid surface.

As the microwave source we used a Siemens BWO 60 backward wave tube (Fig. 8) capable of emitting about 50 mW at any frequency in the range 40 to 60 GHz. This oscillator was controlled by a Micro-Now 702/703 C power supply connected to an Alfred 650 sweep unit. This combination resulted in a short term frequency stability of better than ± 1 MHz. This limit was established by observing an 8 MHz wide beat spectrum resulting from fifth order harmonic mixing with an X-band klystron oscillator (US Army TS-35A/AP), the latter having a short term frequency stability of about

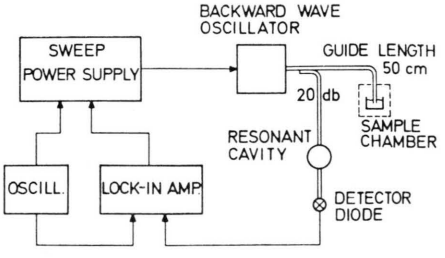

Fig. 8. Schematic diagram of microwave system.

± 1 MHz. These measurements were taken with a Microtel MSR-903 receiver capable of spectrum analysis from 0.03 to 18 GHz with a specified absolute frequency accuracy of ± 1 MHz.

While the frequency of the microwave source could be absolutely determined to within ± 5 MHz in this way, an independent check was made using a cavity calibrated at 20 °C (Hitachi F 2210). We used it at 31 °C. Exact temperature correction curves were not available. Taking the temperature drift indicated by the supplier of about 8 MHz/°C we found a discrepancy of 15 MHz, so that we could expect that our frequency was accurate within about 20 MHz on an *absolute frequency scale.*

For the irradiation experiments we were interested in a high long-term stability, and, in addition, in a high resettability, *i. e.*, accuracy on a relative scale of the frequency. For this purpose we decided to use the calibrated cavity as our frequency standard. To avoid temperature drifts we enclosed the cavity in a double-walled styrofoam box and kept its temperature constant at 31 ± 0.05 °C. The frequency was then taken as the frequency read from the dial minus 88 MHz to correct for the deviation from 20 °C. The BWO frequency was then locked to the cavity by using a feedback loop. This employed a 1 kHz frequency modulation of the BWO with a peak-to-peak amplitude of 1.5 MHz. The response of the cavity resonance (7 MHz FWHM) was synchroneously detected with a PAR 124 A lock-in amplifier and directly fed back into the FM modulation input of the BWO power supply. While for a given setting the long term stability was ± 1 MHz, the total uncertainty was ± 3 MHz from the mechanical resettability of the cavity limiting the accuracy of the *relative frequency scale.*

Experimental Results

In an early stage of these experiments we established the reproducibility of the extinction curves (Fig. 7) without microwave irradiation. Two characteristic numbers were taken from each plot: By graphically fitting a straight line according to $E \sim \exp[\mu \cdot (t - t_A)]$, where t is the time, we obtained the growth rate μ and the "lag time" t_A.

The growth rate μ was found to be reproducible with a small scatter of $\pm 3\%$. A clear temperature dependence resulted as shown as the full line in Fig. 9 a. On the other hand a substantial variation of $\pm 30\%$ was observed for t_A. From a series of specific experiments not reproduced here [7] we know that these fluctuations are correlated with the starting conditions of each growth experiment, mainly the volume of the cells on the Agar plates.

The stirred, unirradiated control culture of about 97 cm³ at 32 ± 1 °C consistently grew up somewhat faster than the culture in the sample cuvette, when no irradiation was applied. After 11 hours the extinction was 1.2 ± 0.1 times higher. This can originate from a decrease in t_A by 8% or an increase in μ by 5%, but probably both contribute. Different environmental factors like cuvette size or stirring method may be the reason.

In order to establish the microwave irradiation effects, we carried out 67 growth experiments during a period of 90 days. Microwave irradiation, if applied, persisted for the full time of each extinction recording. The results of five of these experiments were discarded because the growth rate was very small, in the sample as well as in the control cuvettes probably due to bacterial infection or chemical poisoning. The other experiments are listed in chronological sequence along with their main results in Table I. For the first quarter of these experiments the frequency uncertainty was somewhat larger because the feedback loop of Fig. 8 was not closed; instead, the output of the lock-in amplifier was recorded as a control of frequency stability. The microwave power is not given for the first half of the experiments because a different cuvette holder

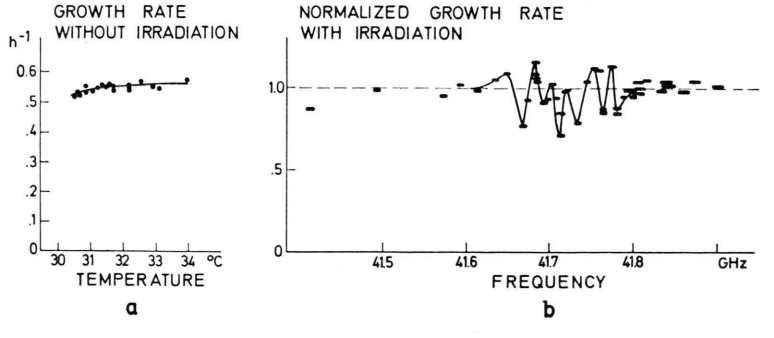

Fig. 9. a. Temperature dependence of the growth rate in the absence of microwave irradiation. The curve was established before the measurements of the additional data points which are taken from Table I.

b. Frequency dependence of the growth rate normalized to eliminate the temperature effect. Width of data shows relative frequency uncertainty; a systematic offset up to ± 20 MHz is possible for the absolute scale. The solid curve is drawn to smoothly connect all data in the central portion of the spectrum.

Table I. Conditions and results of growth experiments in chronological order. Exact quantitative measurements of the power absorbed by the sample were taken in the second half of the measurements only; "+" indicates power monitoring without calibration (orders of magnitude were the same as for the second half).

Experiment number	Frequency [MHz]	Temperature T [°C]	Absorbed microwave power L [mW]	Normalized growth rate μ	Lag time t_A [h]	Starting concentration $\times 10^5$ cm^{-3}
1	—	31.7		0.98	—	3.4
2	—	31.6		1.02	3.4	3.0
3	41838±7	31.1	+	1.02	3.1	3.4
4	41832±6	32.4	+	0.97	3.3	3.3
5	41840±8	33.5	+	1.01	3.0	3.6
6	41860±6	33.8	+	0.97	3.5	3.3
7	41873±6	33.4	+	1.03	3.45	3.2
8	41898±6	32.7	+	1.00	3.25	3.6
9	—	32.2		0.97	3.2	2.9
10	41804±7	33.7	+	0.96	3.0	3.6
11	41806±6	33.9	+	0.99	3.3	3.2
12	—	31.5		1.01	3.35	3.4
13	—	32.9		0.98	3.3	3.5
14	41804±6	33.1	+	1.03	3.5	3.5
15	41793±5	34.0	+	0.98	3.7	3.8
16	41779±4	33.0	+	0.87	4.15	3.7
17	41779±4	34.0	+	0.84	3.4	3.5
18	41634±3	32.3	+	1.04	3.5	3.8
19	—	30.6		0.99	4.0	3.7
20	41613±3	31.1	+	0.98	4.2	3.0
21	—	34.0		1.00	—	3.6
22	41593±3	32.6	+	1.01	3.6	2.8
23	41573±3	33.0	+	0.95	3.0	3.6
24	41648±3	32.7	+	1.08	3.4	3.5
25	—	32.2		0.99	3.5	3.1
26	41703±3	33.7	+	1.02	3.4	3.1
27	—	31.4		1.02	3.3	4.3
28	41683±3	32.9	+	1.04	3.4	3.6
29	—	31.1		1.00	3.5	3.5
30	41693±3	32.4	+	0.91	3.5	3.2
31	41762±3	32.2	+	0.85	4.0	3.7
32	—	30.7		0.98	3.8	3.5
33	41762±3	32.1	+	0.86	3.5	3.5
34	41718±3	32.1	+	0.98	4.4	3.3
35	—	30.6		0.98	4.2	3.2
36	—	32.6		1.02	3.7	3.3
37	41672±3	32.6	+	0.92	4.4	4.0
38	—	33.1		0.97	—	3.8
39	41722±3	32.4	17	0.98	4.0	3.7
40	41682±3	32.3	17	1.15	3.8	3.1
41	—	31.3		1.01	4.1	4.0
42	41752±3	32.6	13	1.11	3.9	4.2
43	41743±3	32.7	22	1.03	3.9	3.6
44	41492±3	32.1	23	0.98	4.2	3.5
45	—	30.9		1.03	3.8	4.2
46	41412±3	32.1	26	0.87	4.0	4.5
47	—	31.7		1.00	—	3.7
48	41757±3	32.5	14	1.10	4.05	4.2
49	—	32.2		0.99	—	3.6
50	41697±3	33.1	19	0.92	3.6	4.5
51	41682±3	33.1	27	1.06	—	4.5
52	41667±3	32.0	18	0.77	4.1	3.0
53	41682±3	31.8	19	1.07	—	3.0
54	41732±3	32.1	14	0.78	4.6	4.0
55	41712±3	31.7	23	0.71	—	4.0
56	—	30.9		0.99	4.6	2.5
57	41787±3	31.8	11	0.94	—	2.3
58	41772±3	31.4	15	1.12	—	2.3
59	41812±3	32.2	17	1.03	—	3.6
60	41797±3	32.4	22	0.94	—	3.6
61	41707±3	30.9	22	0.93	—	4.5
62	41712±3	31.8	17	0.82	—	4.5

was used the heat conduction of which had not been calibrated. The growth rate μ of each experiment was normalized by dividing by the growth rate without irradiation at the appropriate temperature, taken from the solid curve in Fig. 9 a.

All 62 growth rates are shown as data points either in Fig. 9 a or 9 b depending on whether they represent experiments without or with microwave irradiation. The results are very clear: whereas for the unirradiated case the data lie within a small scatter of $\pm 3\%$ from the mean value, at a given temperature, the irradiation is seen to produce large effects up to 29%.

Discussion

The striking irradiation effects on the growth rate of yeast cells can not be explained by a simple thermal effect. This is seen from the weak temperature dependence of the growth rate in Fig. 9 a, together with the fact that the sample temperature T was in the range 30.5 to 34 °C for all experiments (Table I). Also, no correlation is seen in Table I between absorbed microwave power and normalized growth rate. Furthermore, even if the cells were assumed to absorb microwaves much more strongly than water, heat conduction into the surrounding water would hinder any significant overheating. We estimate 50 μs as a characteristic temperature equilibration time between yeast cells, 5 μm in diameter, and water. Even if the cells were to absorb the whole of the microwave power in a surface layer 0.2 mm thick an overtemperature of only 0.2 °C would result. The partial volume of the cells in the growth medium is however only about 0.1%. Water can be considered the dominant absorber of the microwaves as it exhibits strong Debye-like absorption with a constant power penetration depth of 0.2 mm for the frequency range of Fig. 9 b [10].

Apart from the magnitude of the microwave effects in Fig. 9 b the most important result seems to us the pronounced dependence on frequncy. The full line drawn through the data points reveals a multiplet of resonances between 41.64 and 41.79 GHz, each of them having a resonance linewidth of only about 10 MHz. Of course these data points are not corrected for the different irradiation powers used, since nothing is known about the power dependence. With a total variation in power over a factor of 3 for all points, the general shape of the curve would not drastically change even if a linear power dependence was assumed. Clearly a better resettability of the frequency is necessary to establish the power dependence of the effect.

Notice also that the microwave intensity widely varies within our sample. In a typical case we have measured 20 mW of microwave power being absorbed in the sample. We thus have 2 mW/cm² as an average intensity entering through the teflon/water surface. Locally this value may be up to a few times higher through interference effects. Due to the strong absorption of water, the intensity exponentially decays from the surface with a characteristic length of 200 μm. Thus about 9% of the sample is exposed to more than 1 mW/cm² and about 35% to more than 0.1 mW/cm². With the strong motion induced by the stirrer, all cells can be thought of as passing through high and low intensity regions and through the photometer beam. Possibly much stronger growth changes could be expected if all cells stayed exposed to the high intensity.

Let us assume that microwave intensities as low as 0.1 mW/cm² are sufficient for inducing the changes observed in our experiments, and that the effect does not increase at higher intensities. Then nearly all cells in our cuvette contribute equally and we can calculate a lower limit of 20% for the change of the growth rate per cell in the observed resonances. After 10 hours of growth this leads to a change in the multiplication factor by $\exp(2\,\mu) = 3$ taking $\mu = 0.55\ \mathrm{h}^{-1}$, *i. e.* the resonant microwave irradiation has either decreased the cell density to 33% or increased it to 300% relative to an unirradiated control. Thus the effects measured here are at least as strong as those in refs. 2 – 4.

Conclusions

Our results confirm the existence of resonant influences of coherent millimeter microwaves on biological properties. They go much further than the experiments reported in refs. 2 – 4 in revealing the extreme narrowness of the response. This clearly poses most interesting physical questions. Apart from these, a vast and unforeseeable range of applications must be expected. It seems appropriate to recommend strict precautions against possible safety hazards from millimeter microwaves.

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