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ORIGINAL PAPER

# Effect of glycerol and dihydroxyacetone concentrations in the culture medium on the growth of acetic acid bacteria *Gluconobacter oxydans* ATCC 621

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**Abstract** Bacteria of the species *Gluconobacter oxydans* are applied in the industrial production of dihydroxyacetone (DHA) via glycerol oxidation. The major problem of this biotransformation involves process inhibition by substrate and/or product. Improper initial concentration of glycerol and increasing DHA concentration may inhibit the metabolic activity of bacterial cells and impede further course of the reaction. An attempt was, therefore, undertaken in this study to determine which concentrations of glycerol (30, 50, 70, 100 g $L^{-1}$) and DHA (10–100 g $L^{-1}$) may inhibit the growth of acetic acid bacteria of *G. oxydans* ATCC 621 species. Cultures of this strain were run in the Bioscreen C MBR apparatus on experimental culture media with various initial concentrations of glycerol and DHA. Analyses were also carried out to examine the impact of pH (5.0, 7.0, 8.0) of glycerol-containing culture media on cell growth of the analyzed strain *G. oxydans*. None of the applied substrate concentrations was inhibiting cellular divisions of *G. oxydans* bacteria. The initial glycerol concentrations that enabled rapid cellular divisions reached 50 g $L^{-1}$ in the medium with pH 5.0 (coefficient of specific growth rate $\mu = 0.0550$) and 70 g $L^{-1}$ in the medium with pH 7.0 ($\mu = 0.0556$). DHA was shown to inhibit the mitotic activity of *G. oxydans* bacteria even at low concentrations (20–30 g $L^{-1}$), whereas at the concentration of 70 g $L^{-1}$, it made cell divisions impossible. The applied pH values of the culture media did not inhibit the growth of *G. oxydans* strain.

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## Introductory remarks

In the global industry, acetic acid bacteria of the *Gluconobacter oxydans* species are applied for, among other things, the production of dihydroxyacetone (DHA) via glycerol biotransformation [9, 24].

Dihydroxyacetone (DHA) is a ketotriose used in the food industry as a sweetener in products for diabetic patients [26] and as a dietary supplement for sportsmen [26, 33]. DHA has also been applied in cosmetology (active ingredient of tanning lotions), pharmacy (intermediate compound in drugs production), and medicine (treatment of vitiligo, antidote to cyanide-induced intoxications, component of hemorrhage arresting biomaterials) [8, 13, 14, 17, 30].

In *G. oxydans* cells, glycerol biotransformation to DHA is catalyzed by glycerol dehydrogenase (GlyDH, EC 1.1.99.22) that is bound with the cytoplasmic membrane. The reaction proceeds in the periplasmic space of Gram-negative cells of acetic acid bacteria in a pH range of 5.0–5.5 and is independent of the presence of ATP and NAD [12, 25, 29].

During microbiological synthesis of DHA, the retardation or arrestment of cellular division of *G. oxydans* bacteria is likely to occur, which constitutes the major drawback of this method [23]. Proliferation of acetic acid bacteria may as well be inhibited by stress induced by inappropriately selected initial concentration of glycerol (over 50 g $L^{-1}$) and/or increasing concentration of DHA (60–80 g $L^{-1}$) [16, 23, 26, 32, 36]. The cellular mechanisms as well as concentration of glycerol and DHA responsible for this inhibition have not been explicitly identified yet [16].

In the past, scientists tried to develop methods that would enable abolishing the undesirable inhibitory effect [5]. So far, studies have mainly been focused on the determination of the optimal composition and pH of the culture medium [38], process conditions, and degree of reaction medium aeration [18, 35, 36].

In recent years, some works have appeared [13, 15, 20, 22] that addressed glycerol biotransformation to DHA by genetically modified strains of *G. oxydans*. The modifications consisted in, among other things, designing strains capable of GlyDH overproduction, which ensured higher (compared to parental strain) concentrations of the product. However, the effect of inhibition still occurred (though it was lesser compared to the wild strain) and was disturbing the course of biotransformation.

Contemporarily, DHA is produced exclusively with the biotechnological method using free cells of *G. oxydnans*. In the industrial production of DHA via glycerol biotransformation by *G. oxydans,* product's concentration in the reaction medium needs to be continuously monitored and kept at a level below 50 g $L^{-1}$. Biotransformation is run in bioreactors with continuous feeding of the glycerol-containing medium and continuous reception of the post-reaction mixture with DHA [16].

The global demand for DHA is successively increasing, and its new application possibilities are still emerging [10, 17, 19, 37]. It seems advisable, therefore, to search for novel solutions to attenuate or eliminate the problem of inhibition accompanying glycerol biotransformation to DHA. One of such solutions may be the determination of doses of glycerol and DHA in the reaction medium that would inhibit the metabolic activity of *G. oxydans* cells.

The aim of this study was to determine the concentrations of glycerol and DHA that inhibit mitotic divisions of acetic acid bacteria *G. oxydans* ATCC 621. The effect of pH value of culture medium on the growth of this strain cells was examined as well.

## Materials and methods

### Strain and culture conditions

Experiments were conducted with *G. oxydans* ATCC 621 strain of acetic acid bacteria, originating from the American Type Culture Collection, University of Boulevard, Manassas, United States. The strain was inoculated every 30 days onto slant medium containing (g $L^{-1}$) yeast extract 5 (Avantor™ Performance Materials), peptone 3 (Avantor™ Performance Materials), mannitol 25 (Avantor™ Performance Materials), and agar 15 (Avantor™ Performance Materials). For biomass proliferation, a pure culture of *G. oxydans* was transferred into the inoculating culture medium containing (g $L^{-1}$) yeast extract 30 and ethyl alcohol 20 and having pH 5.0. The culture was incubated at 28 °C for 24 h (to obtain $OD_{600nm}$ about 0.7 corresponding to 0.5 g of wet biomass) on a reciprocating shaker (200 cyclesmin$^{-1}$, Edmund Büchler SM-30 Control).

Strain cultures were run in Bioscreen C MBR apparatus in control and experimental culture media. The Bioscreen C MBR apparatus (Oy Growth Curves Ab Ltd. Helsinki, Finland) is designed for microbiological tests that are based on the measurement of optical density (OD) of a liquid medium during the culture of microorganisms. It enables simultaneous running and monitoring of 200 microcultures, placed in incubation cassettes with a volume of 0.4 $cm^3$ in a temperature range of 1–60 °C.

The experiment was carried out in culture media with active acidity of 5.0 that is optimal for the metabolic activity of most species of acetic acid bacteria [31]. Analyses were also conducted for culture medium with pH 7.0 that ensured a high activity of GlyDH and for culture medium with pH 8.0 that stabilized this enzyme [1, 25].

The study investigated various concentrations of glycerol (30, 50, 70, or 100 g $L^{-1}$) and DHA (10, 20, 30, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 g $L^{-1}$) on the growth of *G. oxydans*.

The control medium contained only (g $L^{-1}$) yeast extract 30 and ethyl alcohol 20 (pH 5.0).

All media were sterilized at 121 °C for 20 minutes

Sterile control and experimental media were inoculated with 1 % v/v strain of *G. oxydans* bacteria originating from a 24-h inoculating culture. Shaken culture was run at a temperature of 28 °C, for 180 h. According to the producer's recommendations, a broadband filter ($\lambda$ = 420–580 nm) was selected for OD measurements. The OD of the culture was measured every hour. Strain cultures in a selected medium were carried out in three parallel replications.

### Experimental dates

Experimental dates were analyzed based on the plots of a correlation between culture OD and culture duration, made in Excel sheet. The coefficient of specific growth rate ($\mu$) in time ($t$) was calculated from the formula: $\mu(t) = (\ln OD_f - \ln OD_i)/(t_f - t_i)$, where $OD_f$—final OD in the log phase, $OD_i$—initial OD in the log phase, $t_f$—time of log-phase termination, and $t_i$—time of log-phase onset [39].

### Statistical analysis

Statistical analysis was made for more than 22 700 obtained results. Standard deviation ranged between 0.002–0.017.

## Results and discussion

### Effect of active acidity and glycerol concentration on the growth of *G. oxydans* ATCC 621 bacteria

The OD values obtained in the experiment allowed calculating the coefficient of the specific growth rate of *G. oxydans* cells ($\mu$) and determining the length of the adaptation phase (lag) and intensive divisions phase (log). Respective results were presented in Table 1.

Figure 1 presents curves of a correlation between OD of *G. oxydans* culture in control and experimental media at pH 5.0 and diversified concentrations of glycerol.

Optical density (OD) measurements of *G. oxydans* culture run in the control medium (without glycerol) at pH 5.0 indicate a short 5-h phase of cells adaptation to the proposed environmental conditions (Table 1). In the control medium, the logarithmic phase lasted 10 h and the highest OD value reached 0.385 (Table 1). The coefficient of specific growth rate reached 0.0618, and its value (compared to other $\mu$ values presented in Table 1) showed the capability of *G. oxydans* strains for rapid cellular divisions in the proposed conditions.

The course of the growth curves of *G. oxydans* bacteria in the culture media with pH 5.0 and increasing concentrations of glycerol 30, 50, or 70 g $L^{-1}$ was initially similar (Fig. 1). The logarithmic phase spanned from 10 to 15 h (Fig. 1). Within the next 20 h, differences were observed in the OD of these culture. After 50 h of culture run at pH 5.0, the OD of culture media with glycerol concentration of 30 or 50 g $L^{-1}$ reached ca. 0.3 and did not change till the end of the experiment (Fig. 1).

In the culture medium with pH 5.0 and the lowest applied glycerol concentration (30 g $L^{-1}$), the rate of

**Table 1** Effect of glycerol addition in the experimental media on the coefficient of specific growth rate ($\mu$), length of lag phase, length of log phase, and the highest value of OD

| | | $\mu$ ($h^{-1}$) | Length of lag phase (h) | Length of log phase (h) | Initial OD in log phase | Final OD in log phase |
|---|---|---|---|---|---|---|
| Control medium, pH 5.0 | | 0.0618 | 5 | 10 | 0.208 | 0.385 |
| pH | Glycerol conc. (g $L^{-1}$) | | | | | |
| 5.0 | 30 | 0.0373 | 10 | 10 | 0.208 | 0.320 |
| | 50 | 0.0550 | 10 | 10 | 0.199 | 0.345 |
| | 70 | 0.0450 | 10 | 15 | 0.190 | 0.373 |
| | 100 | 0.0391 | 15 | 20 | 0.187 | 0.409 |
| 7.0 | 30 | 0.0379 | 5 | 15 | 0.191 | 0.337 |
| | 50 | 0.0451 | 8 | 12 | 0.193 | 0.303 |
| | 70 | 0.0566 | 8 | 14 | 0.185 | 0.365 |
| | 100 | 0.0357 | 10 | 19 | 0.180 | 0.355 |
| 8.0 | 30 | 0.0467 | 4 | 11 | 0.177 | 0.296 |
| | 50 | 0.0520 | 7 | 10 | 0.182 | 0.306 |
| | 70 | 0.0443 | 13 | 12 | 0.188 | 0.320 |
| | 100 | 0.0143 | 7 | 43 | 0.179 | 0.331 |

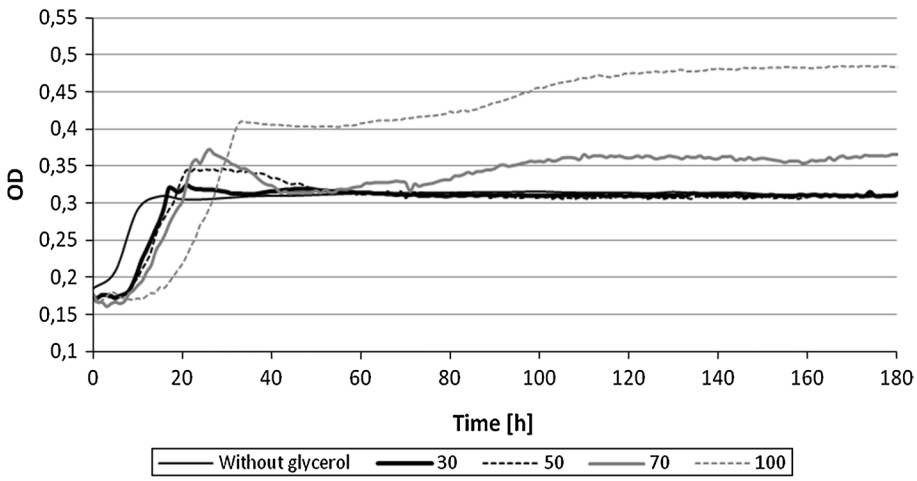


**Fig. 1** Changes in the optical density of *G. oxydans* cells culture in control and experimental media with the addition of glycerol (30, 50, 70, 100 g $L^{-1}$), pH 5.0

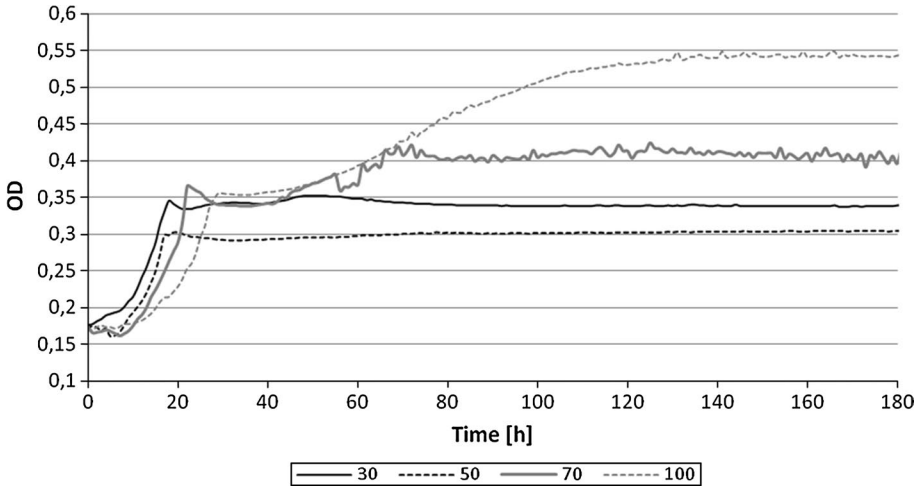


**Fig. 2** Changes in the optical density of *G. oxydans* cells culture in the experimental culture media with the addition of glycerol (30, 50, 70, 100 g $L^{-1}$), pH 7.0

*G. oxydans* cells growth was the lowest, which was indicated by the lowest value of $\mu$ coefficient ($\mu = 0.0373$) in this variant of culture. Probably, the low dose of glycerol (30 g $L^{-1}$) in the medium and its active acidity of pH 5.0 directed the metabolism of *G. oxydans* bacteria toward the activation of glycerol dehydrogenase and not toward biomass production [12, 16, 25, 26]. Glycerol applied in a dose of 50 g $L^{-1}$ initiated cellular division after 10 h of culture incubation, and in this variant, the coefficient of specific growth rate reached $\mu = 0.0550$ and was the highest for all cultures run in culture media with pH 5.0. These observations confirmed earlier literature data [23, 26, 38], according to which glycerol concentration in the medium being optimal for the growth of acetic acid bacteria at pH 5.0 should reach 50 g $L^{-1}$. A successive increase in substrate content (70 or 100 g $L^{-1}$) in the medium with pH 5.0 caused a decrease in the value of $\mu$ coefficient compared to the medium fortified with glycerol at 50 g $L^{-1}$. The applied higher concentrations of the substrate (70 and 100 g $L^{-1}$) probably had a negative impact on the metabolic activity of the analyzed bacteria and enforced longer adaptation (10–15 h) of cells to environmental conditions (Table 1).

In the culture medium with pH 5.0 and glycerol concentration of 100 g $L^{-1}$, a tangible increase in the OD value occurred between the 60th and 120th hour of the culture (OD = 0.400 and 0.480, respectively, Fig. 1). Most likely, part of glycerol was earlier oxidized to DHA, which constituted an easily available source of carbon to *G. oxydans* bacteria and could be utilized for biomass growth [34].

Earlier investigations [23, 26] have shown that glycerol content in the production medium deemed indispensable for the proper course of glycerol biotransformation to DHA should fit within the range of 20–50 g $L^{-1}$. Its higher concentration may lead to the deterioration of acetic acid bacteria growth as a result of hyperosomotic stress as well as to reduced reaction yield. As reported by Mishra et al. [25] and Claret et al. [6], at the initial glycerol content in the medium reaching 90–120 g $L^{-1}$, the cellular divisions of acetic acid bacteria were disturbed and DHA synthesis was impossible. The increasing initial concentration of substrate was accompanied by elongating time of reaction that enabled achieving a high concentration of the reaction product [6, 25].

The above results indicate the feasibility of *G. oxydans* growth at active acidity of 5.0 and applied doses of glycerol (30, 50, 70, and 100 g $L^{-1}$). Simultaneously, the increasing concentration of the substrate in the range of 50–100 g $L^{-1}$ resulted in elongation of the adaptation and logarithmic phases of *G. oxydans* strain. This information may be useful in the formulation of culture medium composition for glycerol biotransformation into DHA.

The next stage of the study included the determination of the impact of various glycerol concentrations in culture media with pH 7.0 on the growth of *G. oxydans*. The active acidity of 7.0 was optimal for the activity of glycerol dehydrogenase that catalyzes glycerol biotransformation to DHA [6, 40]. Our earlier investigations [34] demonstrated the feasibility of running the biotransformation not only at pH optimal for *G. oxydans* growth (pH 5.0), but also at pH optimal for GlyDH activity (pH 7.0). However, at the active acidity of 7.0, the yield of the process depends also on the concentrations of the substrate and the product.

The course of *G. oxydans* ATCC 621 growth in the culture medium with pH 7.0 and initial glycerol concentration at 30 g $L^{-1}$ (Fig. 2) was similar to the growth of this strain in the medium with the same glycerol content but at pH 5.0 (Fig. 1). After 20 h, the OD of the culture in the medium with pH 7.0 (glycerol 30 g $L^{-1}$) was at ca. 0.34 (Fig. 2), whereas in the medium with pH 5.0, its value reached ca. 0.32 (Fig. 1). These results suggest that the applied pH values (5.0 or 7.0) had no significant effect on the growth of *G. oxydans* bacteria in the culture media with the lowest substrate addition (30 g $L^{-1}$).

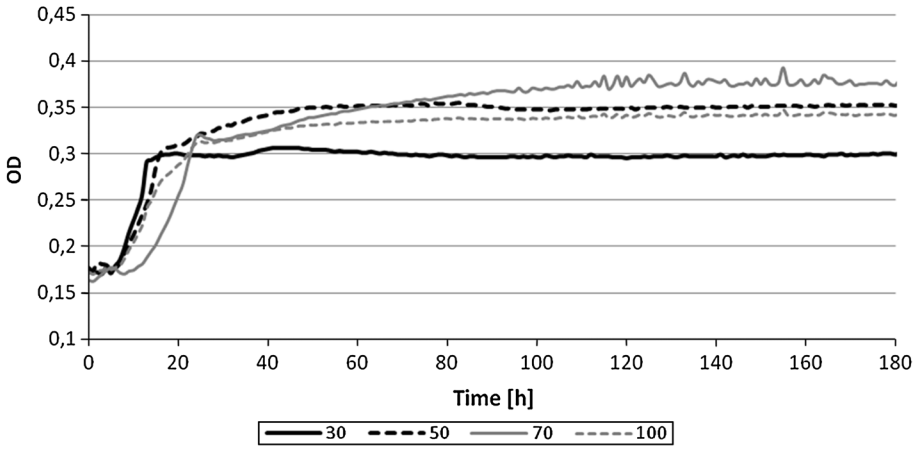


**Fig. 3** Changes in the optical density of *G. oxydans* cells culture in the experimental media with the addition of glycerol (30, 50, 70, 100 g $L^{-1}$), pH 8.0

Experimental variants with pH 5.0 or pH 7.0 and glycerol concentration 50 g $L^{-1}$ (Figs. 1, 2) were characterized by a short phase of cells adaptation to environmental conditions (up to 10 h) and by short logarithmic growth phase (another 10 h). The stationary phase began after 20 h of culture incubation. In that period, the OD value was at ca. 0.345 in the medium with pH 5.0 and glycerol concentration of 50 g $L^{-1}$ and at 0.303 in the medium with pH 7.0 and the same addition of glycerol (Figs. 1, 2).

Cells of *G. oxydans* bacteria placed in the medium with glycerol concentration of 70 g $L^{-1}$ and pH 7.0 needed ca. 8 h to adapt to new environmental conditions. Afterward, a rapid increase was observed in the rate of cellular divisions, which was indicated by an increase in OD value from 0.18 after ca. 15 h of culture to 0.365 after ca. 20 h (Fig. 2).

The measurements of OD of *G. oxydans* culture in the medium with pH 7.0 and the highest initial concentration of glycerol (100 g $L^{-1}$) demonstrate a two-stage logarithmic phase (likewise in the medium with pH 5.0 and glycerol content at 100 g $L^{-1}$, Fig. 1). Between the 15th and the 35th hour of culture, the OD of the suspension increased significantly (from OD = 0.2 to 0.355, respectively); between the 35th and 50th hour, no significant changes were observed in OD values, whereas after 50 h the OD was successively increasing to reach the value of 0.540 after the 130th hour (Fig. 2).

Incubation of *G. oxydans* cultures in the experimental media with pH 7.0 and various initial concentrations of glycerol (30, 50, 70, 100 g $L^{-1}$) enabled shortening the adaptation phase, compared to the cultures run on the same media but at pH optimal for the growth of acetic acid bacteria (pH 5.0), Table 1.

The coefficient of the specific growth rate noted for *G. oxydans* cells cultured in the medium with pH 7.0 and glycerol concentration at 70 g $L^{-1}$ reached 0.0566 and was the highest in this experimental variant (Table 1). The active acidity of 7.0 was not optimal for the metabolic activity of *G. oxydans* cells, although the results achieved clearly showed that the growth of these bacteria was similar to growth observed at pH 5.0 and the same glycerol doses (Table 1). Interestingly, at pH 7.0, the bacterial cells were more quickly adapting to environmental conditions (shorter lag phase) than at pH 5.0 (Table 1). The highest value of the specific growth rate $\mu$ in the medium with pH 7.0 was determined for the cells cultured in the medium with glycerol dose of 70 g $L^{-1}$ ($\mu = 0.0566$), Table 1.

Another stage of the study addressed the impact of various concentrations of glycerol on the growth of *G. oxydans* in culture media with pH 8.0 (Fig. 3). The applied active acidity was stabilizing glycerol dehydrogenase [1, 2, 21]. At pH value in the range of 8.0–10.0 and in the presence of glycerol, cytoplasmic pathway is activated in cells of acetic acid bacteria that leads to the growth of cellular biomass [3, 7].

Cells of *G. oxydans* cultured in the medium with pH 8.0 and the lowest initial concentration of glycerol (30 g $L^{-1}$) reached the stationary phase after 15 h and ever since the OD of the culture was remaining at ca. 0.3 till the end of the experiment (Fig. 3). Changes in the OD of cultures incubated in the medium with pH 8.0 and glycerol concentration of 50 or 100 g $L^{-1}$ were similar throughout the experimental period (Fig. 3). For these cultures, the phase of bacterial cells adaptation to medium conditions lasted 7 h. In the stationary phase, the OD of these cultures was at the level of 0.35 (Fig. 3). Literature data [2, 3, 7] demonstrate that at pH 8.5, glycerol is consumed for the growth of acetic acid bacteria biomass.

No intensive growth of *G. oxydans* cells was observed in the culture media with pH 8.0 and various initial concentrations of glycerol compared to strains grown at pH 5.0 or 7.0 and the same concentrations of glycerol (Figs. 1, 2, 3). In contrast, at the highest active acidity of the medium (pH 8.0, Fig. 3), the OD of *G. oxydans* cultures run at the initial glycerol doses of 70 or 100 g $L^{-1}$, attained the lowest values (0.320 and 0.331 on average) compared to the OD of cultures grown at pH 5.0 (0.373, 0.409) or pH 7.0 (0.365, 0.355) and the same initial concentration of glycerol (Table 1).

Results achieved in the study enable concluding that the highest coefficient of specific growth rate ($\mu$), accounting for 0.0618, was determined for *G. oxydans* cells grown in the culture medium without glycerol addition (Table 1). Results of determinations provided in Table 1 indicate that the initial glycerol concentration in the medium had a greater impact on the growth of *G. oxydans* cells than the active acidity. Regardless of medium pH, values of $\mu$ coefficient were the lowest in the cultures enriched with glycerol doses of 30 or 100 g $L^{-1}$ (Table 1). The growth of *G. oxydans* strain in the medium with pH 5.0 and glycerol concentration of 30 g $L^{-1}$ was characterized by twofold elongation of the lag phase (10 h) compared to the growth of this strain in the control medium. In most variants of this experiment, the increase in the initial glycerol concentration resulted in elongation of both the lag and the log phase of growth (Table 1). Glycerol concentration ensuring the fastest cellular divisions of *G. oxydans* bacteria reached 70 g $L^{-1}$ in the medium with pH 7.0, which was proved by the highest value of $\mu$ coefficient, i.e., $\mu = 0.0566$ (Table 1). In contrast, the lowest value of this coefficient ($\mu = 0.0550$) was determined in the medium with pH 5.0 and initial substrate concentration at 50 g $L^{-1}$. Analyses conducted in this stage of the study show also that bacteria of the species *G. oxydans* are capable to grow at both pH 5.0, which was earlier confirmed in ample research works [11, 31], and at pH 7.0.

## Effect of DHA concentration on the growth of *G. oxydans* ATCC 621 bacteria

The next stage of the study was aimed at determining the effect of various concentrations of DHA on the growth of *G. oxydans* ATCC 621 strain bacteria. Based on the results of earlier analyses (Figs. 1, 2, 3) and the literature data [31], the active acidity of culture media at this stage of the study (pH 5.0) was found optimal for the growth of acetic acid bacteria.

The achieved OD values allowed calculating the coefficient of the specific growth rate of *G. oxydans* cells ($\mu$) and determining the length of the lag and log phases. Respective results were presented in Table 2.

Changes in OD values of the suspension of *G. oxydans* cells in the experimental media with different DHA contents were presented in Figs. 4, 5, and 6.

The study demonstrates that the growth of *G. oxydans* bacteria at low DHA concentrations (10, 20, or 30 g $L^{-1}$) was similar to the cell growth of this strain in the control medium without DHA addition (Fig. 4). The adaptation phase lasted 5 h (Table 1), whereas the logarithmic phase lasted 10 (in the control medium and in the experimental medium with DHA addition at 10 g $L^{-1}$) or 20 h (in the media with DHA doses of 20 or 30 g $L^{-1}$), and the highest OD value reached 0.3 on average (Table 2). The highest coefficient of specific growth rate accounted for 0.0618 in the control medium and for 0.0413 in the experimental medium with DHA concentration of 10 g $L^{-1}$ (Table 2).

Differences in OD values of particular cultures became noticeable when the initial concentration of DHA in the experimental medium reached 40 g $L^{-1}$ (Fig. 4). In this variant, the OD of the culture at the final phase of logarithmic growth was 0.270. In the same time, OD assayed for cultures grown in the media with DHA content of 10, 20, or 30 g $L^{-1}$ reached 0.29 on average (Fig. 4). The value of specific growth rate coefficient in the media with DHA addition at 20 or 30 g $L^{-1}$ reached 0.0231 and 0.0225, respectively (Table 2). In the experimental media with DHA doses from 40 to 70 g $L^{-1}$, the highest value of specific growth rate accounted for 0.0150 (at DHA concentration of 60 g $L^{-1}$) and the lowest one for 0.0100 (at DHA concentration of 70 g $L^{-1}$, Table 2).

The increasing dose of DHA in the culture media (55, 60, 65, 70 g $L^{-1}$) was accompanied by decreasing values of OD of the cell suspension (0.250, 0.243, 0.218, and 0.174, respectively) (Fig. 5). These observations confirm

**Table 2** Effect of DHA addition in the experimental media on the coefficient of specific rate growth ($\mu$), length of lag phase, length of log phase, and the highest OD value

| DHA concentration (g $L^{-1}$) | $\mu$ ($h^{-1}$) | Length of lag phase (h) | Length of log phase (h) | Initial OD in log phase | Final OD in log phase |
|---|---|---|---|---|---|
| Control medium | 0.0618 | 5 | 10 | 0.208 | 0.385 |
| 10 | 0.0413 | 5 | 10 | 0.198 | 0.297 |
| 20 | 0.0231 | 5 | 20 | 0.188 | 0.299 |
| 30 | 0.0225 | 5 | 20 | 0.182 | 0.285 |
| 40 | 0.0102 | 10 | 25 | 0.209 | 0.270 |
| 50 | 0.0113 | 15 | 20 | 0.190 | 0.250 |
| 55 | 0.0127 | 10 | 25 | 0.182 | 0.250 |
| 60 | 0.0150 | 10 | 20 | 0.180 | 0.243 |
| 65 | 0.0110 | 10 | 25 | 0.165 | 0.218 |
| 70 | 0.0100 | 10 | 20 | 0.158 | 0.174 |
| 75, 80, 85, 90, 95, 100 | No growth | | | | |

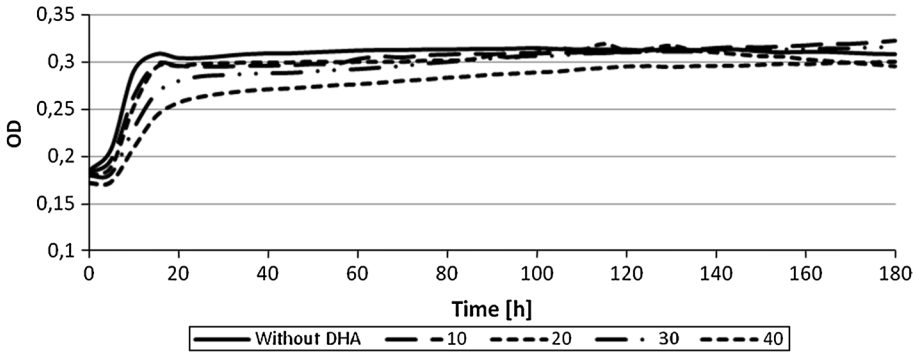


**Fig. 4** Changes in the optical density of *G. oxydans* cells culture in control and experimental media with DHA (10, 20, 30, 40 g $L^{-1}$)

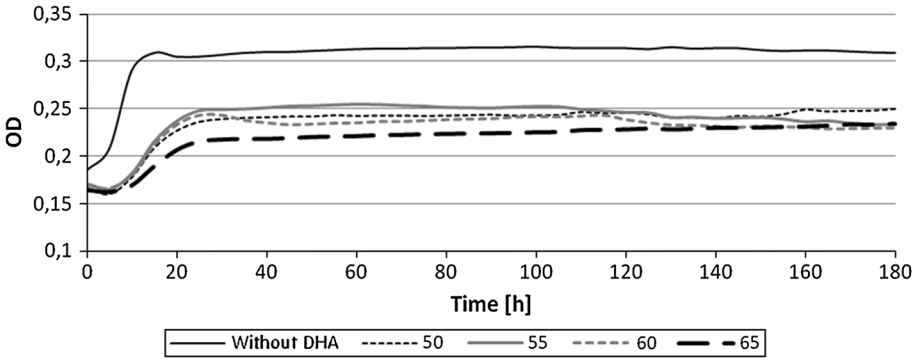


**Fig. 5** Changes in the optical density of *G. oxydans* cells culture in control and experimental media with DHA (50, 55, 60, 65 g $L^{-1}$)

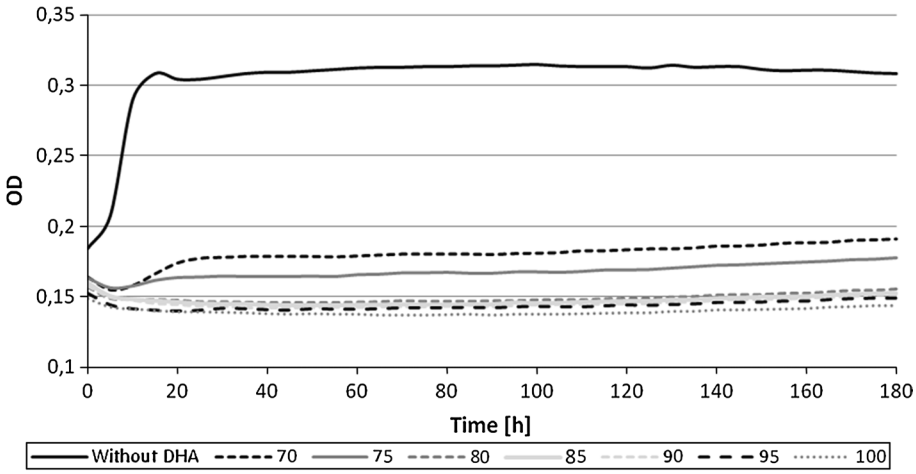


**Fig. 6** Changes in the optical density of *G. oxydans* cells culture in control and experimental media with DHA (70, 75, 80, 85, 90, 95, 100 g $L^{-1}$)

earlier findings [7, 23], according to which the capability of *G. oxydans* cells for divisions diminishes along with an increasing initial concentration of DHA in the culture medium.

Dihydroxyacetone (DHA) concentration exceeding 30 g $L^{-1}$ resulted in elongation of the adaptation phase of *G. oxydans* cells to environmental conditions (compared to the control medium) (Table 2). The coefficient of specific growth rate $\mu$ in the experimental media containing higher than 30 g $L^{-1}$ addition of DHA was about fivefold lower than in the control medium (Table 2).

Figure 6 depicts changes in OD values of *G. oxydans* cultures in the experimental media with DHA addition at 70, 75, 80, 85, 90, 95, or 100 g $L^{-1}$. The presented results show explicitly that the growth of *G. oxydans* bacteria was inhibited at DHA concentrations exceeding 70 g $L^{-1}$ (Fig. 6).

The increasing concentration of DHA in the culture medium was accompanied by a slower increase in OD values (Figs. 4, 5, 6), which probably indicated the arrestment of cell divisions. Similar conclusions were formulated by Claret et al. [7]. Literature data [36] show that the reaction of glycerol oxidation to DHA was significantly impaired

at product concentration of 85 g $L^{-1}$. The growth of acetic acid bacteria was inhibited when the content of resultant DHA in the medium exceeded 67 g $L^{-1}$. This concentration of the product in the reaction medium induced irreversible destruction of cells of acetic acid bacteria. This could be due to the inhibiting effect of DHA on the activity of enzymes of the pentose cycle [4, 6, 25].

In the reported experiment, the lowest value of the specific growth rate $\mu$ ($\mu = 0.0100$) was noted for cells grown on the medium with DHA addition of 70 g $L^{-1}$ (Table 2). The study demonstrates, therefore, that even low doses of DHA present in the culture medium (20, 30 g $L^{-1}$) had a negative impact on the growth of *G. oxydans* strain and that doses exceeding 70 g $L^{-1}$ completely arrested the growth of these bacteria.

## Conclusions

The main drawback of the biotechnological production of DHA via glycerol oxidation by acetic acid bacteria of the *G. oxydans* species is process inhibition by substrate and/or product. The improper initial concentration of the substrate and increasing concentration of the product may inhibit the metabolic activity of bacterial cells.

Glycerol concentration that enabled rapid cell divisions of *G. oxydans* at pH 5.0 reached 50 g $L^{-1}$. In turn, in the culture medium with pH 7.0, the highest specific growth rate of cells was determined at glycerol concentration of 70 g $L^{-1}$, whereas in the medium with pH 8.0, the highest specific growth rate of cells was determined at glycerol concentration of 50 g $L^{-1}$. It was demonstrated that DHA had an inhibiting effect on the metabolic activity of *G. oxydans* bacteria even at low concentrations (20–30 g $L^{-1)}$, and that above 70 g $L^{-1}$, it made cell divisions impossible.

The presented results show explicitly that product concentration which inhibited the metabolic activity of *G. oxydans* cells reached 30 g $L^{-1}$.

Based on the results achieved in the study, it may be concluded that the culture medium for efficient glycerol biotransformation of DHA could contain substrate in the concentration of 30 g $L^{-1}$ and, simultaneously, exhibit active acidity stabilizing GlyDH (pH 8.0). Product concentration obtained under these conditions, i.e., 20–30 g $L^{-1}$, will not inhibit the metabolic activity of the analyzed *G. oxydans* strain.

**Conflict of interest** The authors declare that they have no conflict of interest.

**Compliance with Ethics Requirements** This article does not contain any studies with human or animal subjects.



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