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Article

Novel Silicone-Polyol Antifoam Emulsions: Impact on Foam Control and Physiology of Diverse Microbial Cultures

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Abstract

The selection of an optimal antifoam is critical for efficient fermentation, as industrial agents often have detrimental side effects like growth inhibition, while some can enhance productivity. This study presents a rational approach to developing and screening novel silicone-polyol antifoam emulsions. A key finding was the discovery of selective antibacterial activity in agent 3L10, which strongly inhibited Gram-positive bacteria (especially *Corynebacterium glutamicum*) but not Gram-negative strains. This specificity, likely mediated by interaction with the mycolic acid layer of *C. glutamicum*, highlights the necessity for strain-specific antifoam testing. A comprehensive evaluation protocol—combining chemical design, cytotoxicity screening across diverse microorganisms, determination of minimum effective concentrations (MEC), and validation in model bioreactor fermentations—was established. Through this process, agent 6T80 was identified as a promising candidate. It exhibited low MEC, high emulsion stability, no cytotoxicity, and did not impair growth or recombinant protein production in *B. subtilis* or *P. putida* fermentations. The study concludes that agent 6T80 is suitable for further application in processes involving Gram-negative and certain Gram-positive hosts, whereas agent 3L10 serves as a valuable tool for studying surfactant-membrane interactions. The developed methodology enables the targeted selection of highly efficient and biocompatible antifoams for specific biotechnological processes.

Keywords: fermentation; foam formation; antifoam agents; Gram-positive microorganisms; Gram-negative microorganisms

1. Introduction

1.1. Relevance of Foam Formation in Biotechnological Processes

Foam formation in industrial microbial cultivation becomes particularly important during the scale-up of fermentation processes, where excessive foaming can reduce fermentation efficiency up to complete equipment failure [1]. At present, the global market for defoamers used in fermentation processes is estimated at USD 2.1 billion in 2025 and, with an annual growth rate of 4.6%, will reach USD 3.4 billion by 2035, indicating sustained demand for solutions to this problem [2].

Losses due to uncontrolled foaming during industrial microbial cultivation are estimated to constitute a significant contribution to overall production costs [3]. Production inefficiencies due to foaming arise from decreased substrate-to-product conversion and reduced product per batch [1]. In aerobic processes, the costs of mixing energy, oxygen mass transfer, and cooling exceed 10% of

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product manufacturing costs, and uncontrolled foaming can substantially increase these expenses [4]. Foam formation reduces the effective working volume of the bioreactor and can lead to non-sterile process conditions [5].

Studies applying machine learning to industrial data (half a million instances for 163 batches) showed that optimizing antifoam control achieved a 53% reduction in hourly foam volume, leading to significant improvements in product yield and process economics [6].

A key complication highlighted by research is the severe disruption of the volumetric oxygen transfer coefficient $k_L \times a$ during foaming [7]. In aerobic fermentation processes, adequate dissolved oxygen (DO) transfer is a necessary condition for microbial growth [8]. In passively aerated systems (shake flasks), the situation is quite different. A thin layer of mobile foam can increase the gas–liquid interfacial area, leading to a several-fold increase in $k_L \times a$. However, once a massive layer of stagnant foam forms, this effect reverses: the foam becomes an additional resistance layer to mass transfer, severely impairing oxygen supply [5].

A study of industrial defoamers used in the Brazilian ethanol industry showed that adding these reagents exerts a paradoxically negative impact on the fermentation process itself, even when used for foam control. Industrial defoamers used at concentrations of 60 mg/L led to a 4–6% decrease in the growth rate of *Saccharomyces cerevisiae* cells, and the effect intensified with increasing defoamer concentration. Transcriptomic analysis in the study showed that defoamers induced additional stress responses in yeast cells, including suppression of genes responsible for lipid biosynthesis, especially ergosterol, suggesting changes in membrane composition and increased permeability. Commercial defoamers commonly used in the scientific literature exhibited a better safety profile when applied at the same or even higher concentrations, underscoring qualitative differences among defoamer formulations [9].

In the cultivation of biosurfactant-producing strains such as *Pseudomonas aeruginosa*, which are prolific producers of rhamnolipids, the foaming problem reaches extreme proportions. Rhamnolipids possess an exceptional ability to stabilize foam due to their structure with long hydrophobic fatty acid chains and hydrophilic carbohydrate moieties. During cultivation of such strains, foaming can begin in the exponential growth phase and continue into the stationary phase, creating not only local but systemic challenges for fermentation control. One solution to this issue is the development of strains with modified cell surfaces, in which genes encoding cell-surface structures responsible for hydrophobicity are deleted. Such modified strains exhibited a 46% reduction in cell adhesion to foam, which substantially improved foam fractionation while maintaining target product productivity [10, 11, 12].

1.2. Mechanisms of Foam Formation in Bacterial Cultures

Foam in biotechnological systems is a colloidal system in which the gas phase is dispersed within the liquid phase of the culture broth containing microbial cells and their metabolic products. The process is initiated by intensive aeration, required to maintain sufficient dissolved oxygen for the aerobic growth of bacterial cells, and by mechanical agitation, which fragments large gas bubbles into many smaller ones, increasing the overall gas–liquid interfacial area [13].

The core mechanisms of foam formation during cultivation involve surface-active agents (amphiphilic molecules), including proteins that are naturally produced by microorganisms or released upon their lysis [13, 14]. Polysaccharides and exopolysaccharides also contribute to foam stabilization. Physically, exopolysaccharide fibers can act as emulsion stabilizers by forming a volumetric gel that slows liquid drainage from foam films and prevents bubble coalescence. At the molecular level, negatively charged exopolysaccharides form electrostatic complexes with proteins, enhancing their foaming ability via a mechanism known as electrostatic complexation [15].

The bubble formation rate also critically affects foam stability because it determines the time available for adsorption of proteins and stabilizing molecules onto the surfaces of freshly formed bubbles. A study using a microfluidic bubble-generation system showed that bubble formation time can range from 0.01 to 2.8 milliseconds depending on applied pressure and liquid flow rate. At time

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scales of 0.01–1 millisecond, the effect intensifies with eventual bubble coalescence, making foam size and stability sensitive to operating parameters. This overlap of time scales between bubble formation and protein adsorption explains the observed phenomena of controlled bubble coalescence in practical fermentation systems [16].

1.3. Classification and Mechanisms of Action of Antifoams

In industrial cultivation of microorganisms, antifoams are typically based on polydimethylsiloxane or polyether systems, and their action consists in destabilizing interfacial foam films through rapid entry and spreading over the film and bubble coalescence, while maintaining culture viability and productivity at minimally effective doses [9].

Silicone antifoams include PDMS oils, whose distinctive features are notably low surface tension and high efficacy at low doses [17]; however, their effects on cells and mass transfer processes require control, because overdosing can reduce performance and trigger cellular stress responses. Such effects have been demonstrated for yeasts in ethanol fermentation and in other studies [1, 9].

Polyether antifoams include polypropylene glycol or nonionic ethylene oxide–propylene oxide block copolymers [18]. These molecules disrupt the structured interfacial film and promote bubble rupture, which correlates with their microscopically observable configuration and dynamics at the gas/water interface [19].

Vegetable oils (sunflower, soybean, rapeseed, *etc*.) and their blends with hydrophobic additives should be highlighted, as they suppress foam by penetrating and spreading over the thin foam film and by inducing bubble coalescence [20]. In laboratory tests, ordinary food-grade vegetable oil remains the most accessible and reliable means for promptly quenching foam [20]. The key limitations of oil-based antifoams are the reduction of effective oxygen transfer and the induction of cellular stress responses when overdosed, which inevitably leads to decreased process performance [1]. The composition and dosage of vegetable oils can also alter metabolism and the yield of the target product, as noted for *Streptomyces clavuligerus* when olive and corn oils were added to the medium [21]. At high doses, vegetable oils can complicate downstream processing due to emulsification and increased filtration load; therefore, in practice, minimally effective doses are used with DO control [12, 22].

There are many different types of antifoams, but the silicone type is widely used in the biotechnology industry. Silicone-based antifoaming agents are particularly popular due to their food-grade nature and environmental safety [23]. Silicone-based defoamers are PDMS, which are insoluble in water and exhibit low volatility. These properties result in low spreading coefficients and low dispersion in foaming systems, making them less effective when used in pure form [24]. That's why, silicone-based defoamers contain fine particulate matter, in particular silica SiO_2, which creates a large surface area, and an emulsifier that promotes the distribution of components. According to some authors, highly dispersed, hydrophobic, solid silica SiO_2 is the main active ingredient and must be present for the destruction of foam by silicone defoamers [17, 25].

Based on the literature data and our experience on organic defoamers [26], the appropriate silicone-containing emulsions were selected. This study implements a new approach for a silicone-based defoamers using nonionic surfactants Tween 80 (Tw80) and Laureth-10 (L10) and higher aliphatic fatty alcohol. The prepared emulsions were added directly to the fermentation mixtures.

2. Materials and Methods

2.1. Synthesis of Antifoam Agents

Nonionic surfactants Tween 80 (Tw80) and Laureth-10 (L10) was were purchased from Sigma-Aldrich (USA) and NORCHEM LLC (Nizhny Novgorod, Russia) respectively., and fatty alcohol 2-hexyl-1-decanol (2-HDol, 95%) was purchased from Macklin (China). Organosilicon compounds with cyclic (hexamethylcyclotrisiloxane (D3), octamethylcyclo-tetrasiloxane (D4), decamethylcyclopentasiloxane (D5), dodecamethylcyclohexasiloxane (D6)) and linear structures

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(polydimethylsiloxane with a viscosity of 200 cSt (PDMS200)) were purchased from Shandong Baolongda Industrial Group Co., Ltd. (Weifang, China). Emulsions were prepared using ultra-purified water (18.2 MΩ · cm resistivity at 25°C) (Simplicity® UV, Millipore SAS, Molsheim, France). The structures of the compounds used are shown in Figure 1.

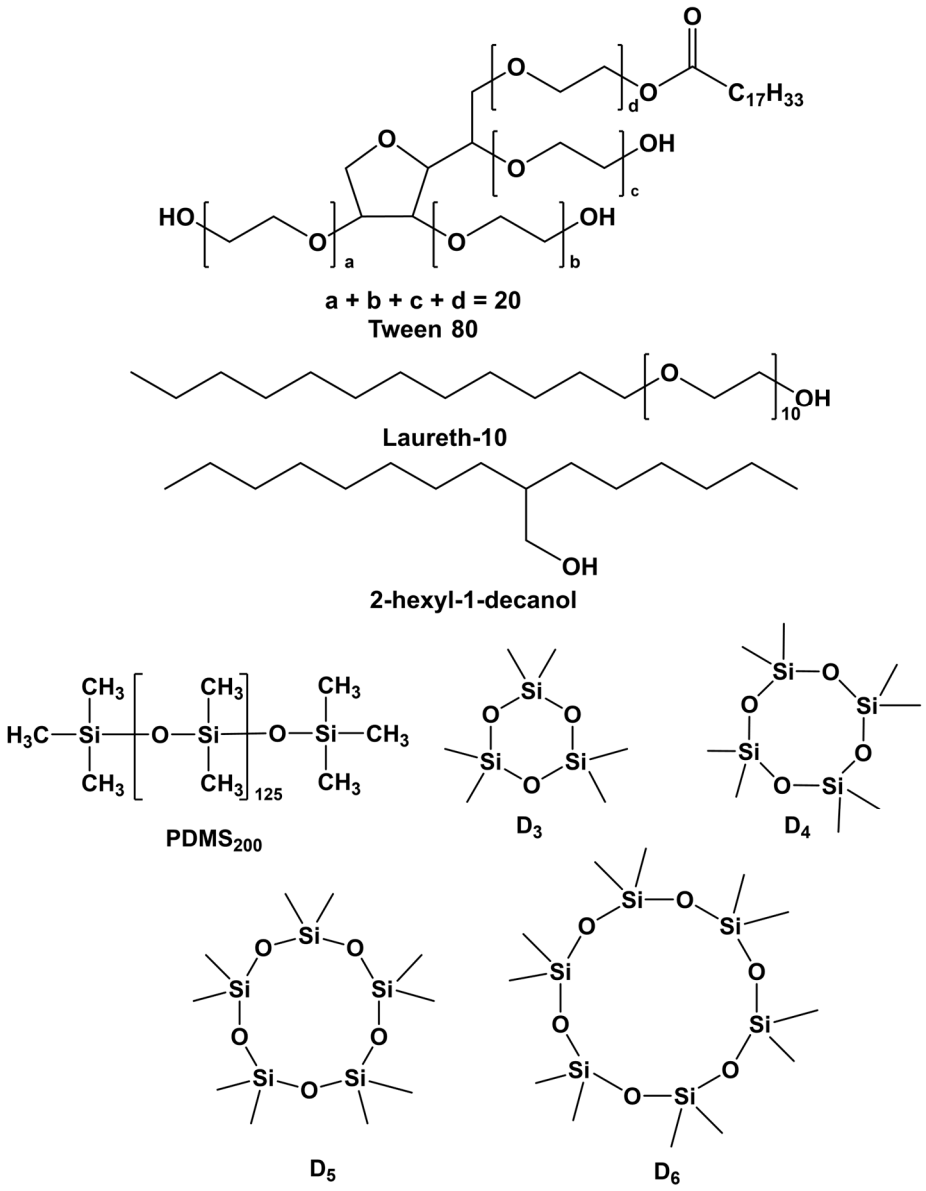

Figure 1. The structures of the compounds.

Emulsions were prepared by mixing the nonionic surfactants Tw80 and L10, $PDMS_{200}$, and 2-HDol in a glass vial at various component ratios. The required amounts of each compound (in wt %) were weighed using an analytical balance DV215CD (Ohaus, Greifensee, Germany). After combining all components, the mixture was homogenized using a ZX3 vortex mixer (VELP Scientifica, Usmate, Italy) for 5 min. Purified water was then added to the system, resulting in an increase in both viscosity and homogeneity of the emulsion. The stability of the emulsions was evaluated visually over time. Emulsions that did not exhibit phase separation for 24 h were considered stable. The component ratios for the different formulations are presented in Table 1.

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Table 1. The component ratios for the different formulations.

Composition	Water	Tw80	$PDMS_{200}$	2-HDol	D_3	D_4	D_5	D_6	D	SiO_2
	wt%									
1T80	25	10	10	35	20	-	-	-	-	-
3T80					-	20	-	-	-	-
3T80-SiO_2			30		-	-	-	-	-	1
4T80			10		-	20	-	-	-	-
4T80-SiO_2					-	20	-	-	-	1
5T80					-	-	20	-	-	-
6T80	25	10	10	35	-	-	20	-	-	1
7T80					-	-	-	20	-	-
9T80					-	-	-	-	20	-
3L10	25	10 (L-10)	10	35	-	20	-	-	-	-

2.2. Inhibition of Growth on Plates

To assess the cytotoxicity of the synthesized antifoams against different groups of biotechnologically relevant microorganisms, the strains listed in Table 2 were selected.

Table 2. Strains used in the experiment.

Strain	Cultivation Medium	Cultivation Conditions	Source Link
Escherichia coli BL21	Luria-Bertani	37°C	[17]
Corynebacterium glutamicum MGMM638	MRS	37°C	[18]
Trichoderma viride MGMMF32	King's B	30°C	[19]
Pseudomonas fluorescens MGMM121	King's B	25°C	[20]
Bacillus subtilis MGMM36	King's B	30°C	[21]
Lactobacillus plantarum MGMM126	King's B	30°C	[22]

The cited sources show that each of these strains can be used either as a cell factory for the production of industrially important enzymes or as an independent biopreparation. Thus, optimizing the cultivation conditions for these strains, including the choice of antifoam, is a relevant task.

The microbial strains were cultivated in suitable liquid growth media at a set temperature for 16 hours. A 100 μL aliquot of culture broth was taken and spread on a Petri dish containing an appropriate agarized nutrient medium. Sterile filter paper discs were then placed on the plate, and 10 μL of antifoam at a concentration of 1% stock solution was applied to each disc. The inhibition zone was assessed after 48 hours of incubation.

2.3. Comparison of the Growth Rate

The resulting cell suspension of the used strains (section 2.2) was diluted with fresh growth medium to an optical density of approximately $OD_{600} \approx 0.1$. Cell cultures were prepared in a volume of 200 μL with the addition of 10 μL of undiluted antifoam mixture (100%) and incubated in 96-well plates (Costar, New York, NY, USA) with continuous shaking for 24 hours at 30°C. The growth rate was assessed by measuring the optical density (OD_{600}) of each culture twice per hour per well at a wavelength (λ) of 600 nm using a spectrophotometer (Feyond A400, Allsheng, China).

2.4. Determination of the Effective Concentration of Antifoams in a Conical Tube

A conical tube (Costar, USA) was filled with 30 ml of KB culture medium and vigorously shaken by hand to establish a normal level of foam formation. Next, we added 20 μL of defoamer and repeated shaking, assessing the foam level, adding 20 μL of defoamer as needed. The effective

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defoamer concentration was considered to be the volume at which the foam level did not exceed 3 mm.

2.5. Comparative Analysis of Culture Growth in Small Parallel Bioreactors

Evaluation of antifoam performance in laboratory fermenters was carried out in two parallel KV-108 bioreactors (Prointech Bio, Moscow, Russia) with a maximum working volume of 2000 ml. The working volume was 1600 ml. To obtain a preculture, a single colony from an agar plate was inoculated into 50 ml of the appropriate nutrient medium and incubated for 16 h at the appropriate temperature with agitation at 180 rpm.

Before sterilization (120°C, 20 min), the reactor was filled with complete nutrient medium and cooled to the operating temperature (25–37°C). For inoculation, 50 ml of the preculture was transferred from a sterile flask into the reactor. During cultivation, pH was maintained at the desired level by adding 0.1 M NaOH or 0.1 M HCl. The dissolved oxygen concentration was maintained above 20% air saturation by adjusting the agitation speed (180–300 min^{-1}). The aeration rate was maintained at 3 $L \cdot min^{-1}$. Culture samples were collected from the reactor 5 min after inoculation and then at 2, 4, 6, 8, 10, 12, and 24 h of the fermentation process. Serial dilutions and plating were performed to determine the number of viable cells (CFU/ml) using an EasySpiral device (Interscience, Saint-Nom-la-Bretèche, France). Colony counting was carried out according to the manufacturer's instructions using the Scan 1200 system (Interscience, France).

2.6. Assessment of the Antifoam Agent's Impact on Glycerol Dehydrogenase Biosynthesis in Pseudomonas putida cells as a Model

To study the effect of a defoaming agent on the efficiency of heterologous protein expression, a model recombinant plasmid system was employed. The construct was created based on the shuttle vector pJem2 [27], into which the gene encoding glycerol dehydrogenase (*gld*A) was cloned under the control of a rhamnose-inducible promoter. The target protein, with a predicted molecular mass of ~38 kDa, exhibits a high expression level, ensuring its reliable detection and visualization within the total cellular protein mixture. A strain of *Pseudomonas putida* LN6160 [12] transformed with the pJem2-gld plasmid was used for this experiment. Target protein expression was induced with 0.2% rhamnose, followed by 16-hour cultivation at room temperature. During subsequent fermentation of this induced culture, the antifoam's effect was determined by measuring the recombinant protein yield.

The conditions for preparing and operating the parallel bioreactors were similar to those described in Section 2.5. Luria-Bertani was used as a nutrient medium. Expression of the target protein was induced by the addition of 0.2% rhamnose, followed by 16 h of cultivation at room temperature. Plant oil and 6T80 were used as antifoam agents. Antifoam addition was performed automatically upon sensor activation. During subsequent fermentation of this induced culture, the antifoam's effect was determined by measuring the recombinant protein yield.

Cultures of 15 ml were pelleted by centrifugation at 5000 x g for 10 min at 4°C. The pellet was thoroughly resuspended in 5 ml Tris-HCl buffer (pH 7.5) to obtain a homogeneous suspension, while keeping the cells on ice. Cells were lysed at 4°C using an ultrasonic homogenizer (Bandelin electronic, Berlin, Germany). Cell debris and large molecular complexes were sedimented in a Gyrozen 2236R ultracentrifuge (Gyrozen, Daejeon, Korea) at 20,000 x g for 30 min at 4 °C. The protein concentration was analyzed spectrophotometrically at 280 nm using a NanoDrop (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Before electrophoresis, the samples (80 μg) were mixed with loading buffer containing β-mercaptoethanol and denatured at 95 °C for 10 min. The protein fractions were analyzed using 12% Tris-glycine SDS-PAGE. Protein bands were visualized by staining the gel with a solution of 2% Coomassie Brilliant Blue R-250 solved in 20% ethanol, 10% acetic acid and 70% of water. The gel was documented with a GelDoc Go Gel Imaging System (Bio-Rad, Hercules, CA, USA).

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3. Results

3.1. Formation of Antifoam Agents

The emulsified form of silicone compounds was prepared using the nonionic surfactants Tw80 and L10, which is widely employed as a stabilizer and emulsifier in the pharmaceutical, food, and cosmetic industries. As a co-surfactant, the long-chain alcohol 2-HDol was selected. Linear polydimethylsiloxane $PDMS_{200}$ and cyclic silicone compounds (D_3, D_4, D_5, D_6, and their mixture) were used as the defoaming agents. The component ratios for the different formulations are presented in Table 1. The resulting emulsions were milky-white in appearance and exhibited moderate viscosity. All formulations demonstrated high stability during storage for more than three months.

3.2. Inhibition of Growth on Plates

In experiments assessing the growth-inhibitory effect of the agents, agent 3L10 demonstrated the formation of inhibition zones on lawns of *Bacillus subtilis* MGMM36 (clear zone 1 mm), *Corynebacterium glutamicum* MGMM638 (clear zone 4 mm), and *Lactobacillus plantarum* MGMM126 (clear zone 3 mm) (Figure 2). All other agents showed no inhibitory effect against any of the tested strains.

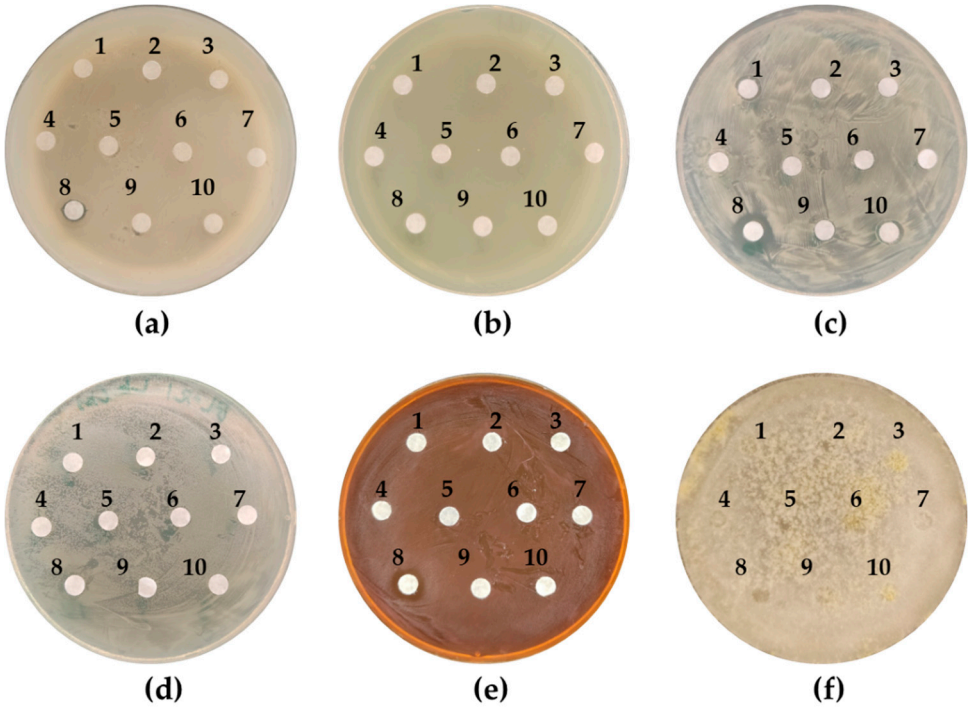

Figure 2. Inhibition of microbial growth on Petri dishes: **(a)** *Bacillus subtilis* MGMM36, **(b)** *Pseudomonas fluorescens* MGMM121, **(c)** *Corynebacterium glutamicum* MGMM638, **(d)** *Escherichia coli* BL21, **(e)** *Lactobacillus plantarum* MGMM126, **(f)** *Trichoderma viride* MGMMF32. 1. 1T80, 2. 3T80, 3. 3T80Si, 4. 4T80, 5. 4T80Si, 6. 5T80, 7. 6T80, 8. 3L10, 9. 9T80, 10. 7T80.

The variation in inhibition zone diameters indicates that the suppression of growth in *B. subtilis*, *C. glutamicum*, and *L. plantarum* follows a concentration-dependent manner. Specifically, *B. subtilis* is inhibited only at high agent concentrations, whereas *C. glutamicum* and *L. plantarum* exhibit sensitivity even at its low concentrations.

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3.3. Assessment of the Effect of Antifoam Agents on the Growth of the Tested Bacterial Strains

We also evaluated the effects of various agents at a fixed concentration (33.7 µg/ml) on microbial growth in liquid medium. Optical density was measured every 1 hour over a 20-hour period (Figure 3). The experiment demonstrated that the growth rates of *P. fluorescens* MGMM121 (Figure 3(b)) and *E. coli* BL21 (Figure 3(d)) remained largely unaffected by all tested agents. Conversely, we observed significant growth inhibition of *C. glutamicum* MGMM638 (Figure 3(c)) in the presence of the antifoam agent 3T80Si. This result contradicts the data obtained from the disk diffusion sensitivity test, where the agents were applied to a bacterial lawn using 3MM paper disks. In that test, the *C. glutamicum* strain showed no sensitivity to this agent (labeled as 3 in the Figure 2(c)). A clear inhibitory effect on *T. viride* (Figure 3(f)) cells was demonstrated for antifoam agent 4T80. However, the optical density of this culture could not be measured reliably due to the plate wells being overgrown with *T. viride* mycelium, thus limiting data interpretation.

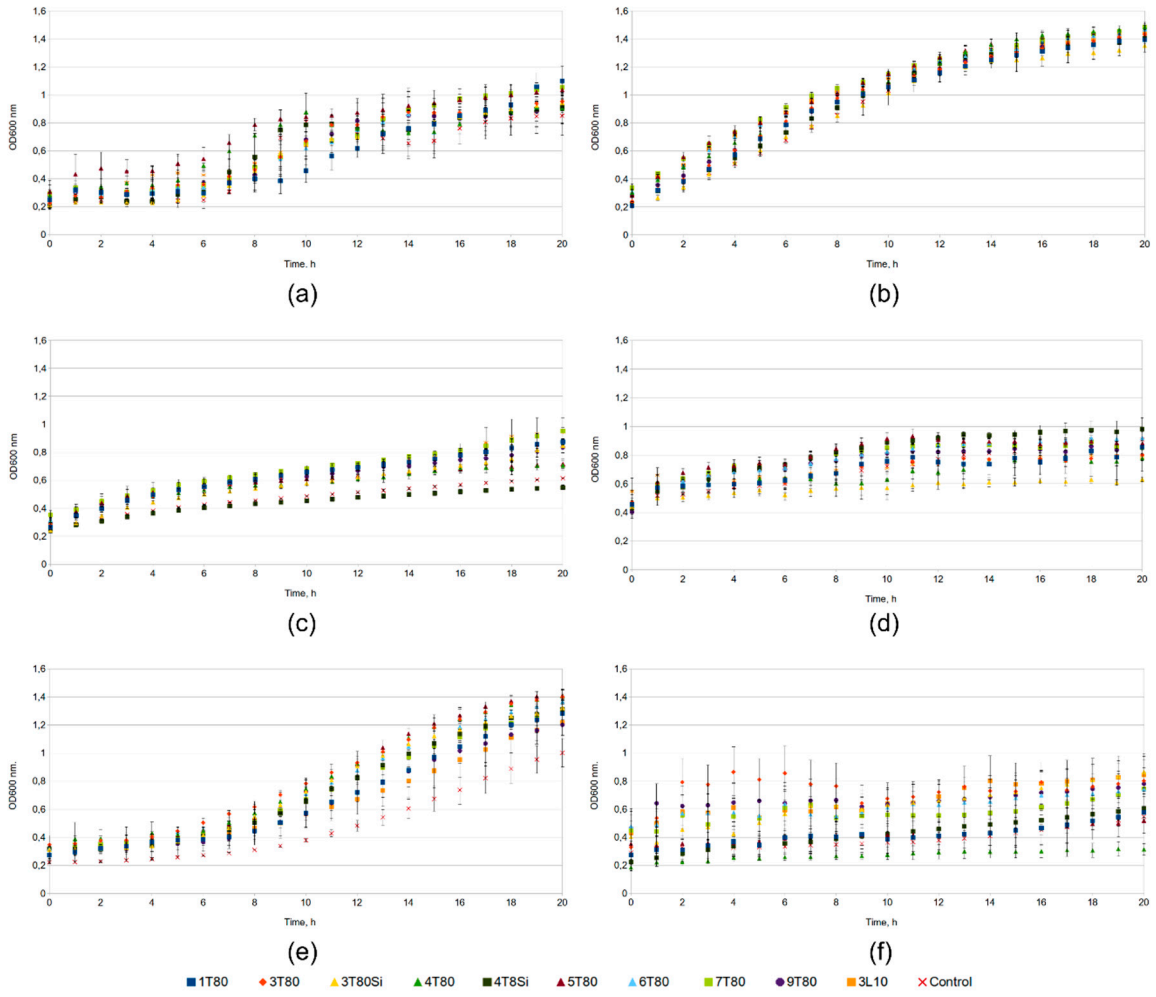

Figure 3. Growth curves of the different strains in the present of antifoam agents **(a)** *B. subtilis* MGMM36, **(b)** *P.fluorescens* MGMM121, **(c)** *C. glutamicum* MGMM638, **(d)** *E.coli* BL21, **(e)** *L. plantarum* MGMM126, **(f)** *T. viride* MGMMF32.

Additionally, the experiment demonstrated that some of the tested agents have a growth-promoting effect on *L. plantarum* MGMM126 and *E. coli* BL21.

3.4. Determination of the Effective Concentration of Antifoams in a Conical Tube

To assess the antifoaming (defoaming) properties of the agents, a shake test was employed using tubes containing the nutrient medium and the test compound. A representative result of this test is

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shown in the figure (Figure 4). When the nutrient medium in conical tubes (Figure 4(a)) was subjected to vigorous shaking, it generated a foam layer that occupied approximately one-third of the tube volume (Figure 4(b)). The addition of the defoamer markedly reduced this foam layer (Figure 4(c)).

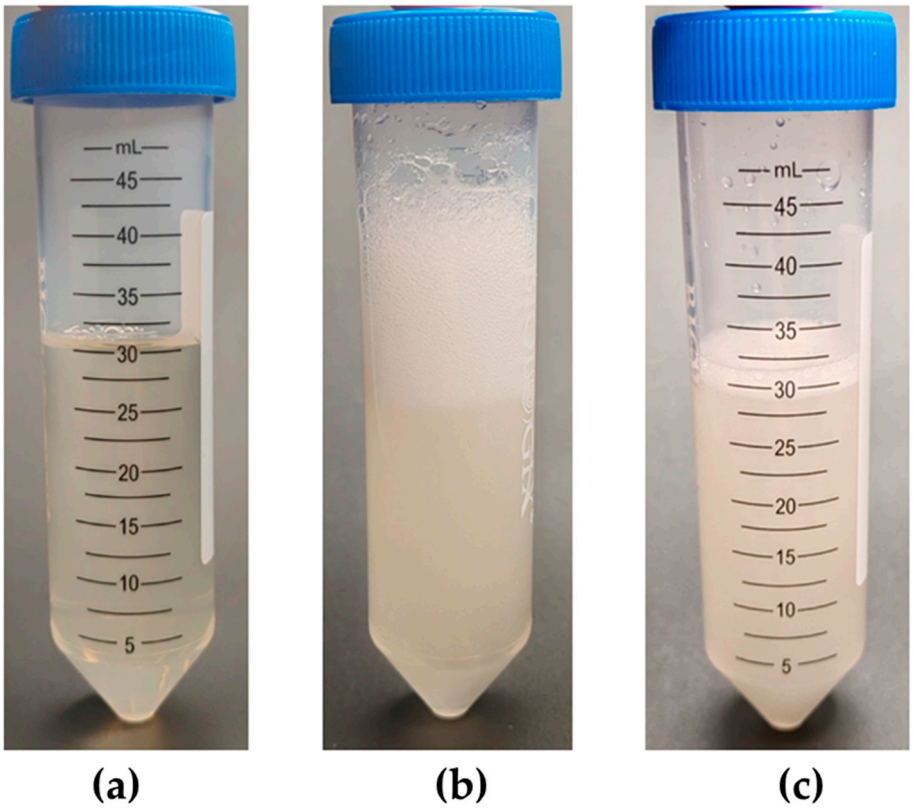

Figure 4. The foam volume of **(a)** LB nutrient medium**, (b)** foam formation upon shaking and **(c)** foam formation upon shaking with added antifoam agent.

The minimum concentration required to achieve complete foam suppression was defined as the minimum effective concentration (MEC) for each defoamer. The results of this experiments are summarized in Table 3.

Table 3. Minimum effective concentration (MEC) of defoamer to suppress foam formation.

Antifoam Agent	Added Volume, µL	Concentration in Solution, % (v/v)
Plant oil	80	0.267
1T80	20	0.067
3T80	40	0.133
3T80-SiO_2	40	0.133
4T80	40	0.133
4T80-SiO_2	40	0.133
5T80	40	0.133
6T80	20	0.067
7T80	60	0.200
9T80	40	0.133
3L10	20	0.067

As a reference, the addition of 80 µL of vegetable oil to 30 ml of medium (final concentration: 0.267 %) was necessary to produce the foam suppression effect shown in Figure 4c. In contrast, the antifoam agents 1T80, 6T80, and 3L10 demonstrated the lowest MEC. A volume of 20 µL per 30 ml

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of medium (final concentration: 0.067%) was sufficient for these agents. Foam suppression required 40 μL (final concentration: 0.133%) for agents 3T80, 3T80-SiO_2, 4T80, 4T80-SiO_2, 5T80, and 9T80. Finally, agent 7T80 showed efficacy at a volume of 60 μL, resulting in a final concentration of 0.200%. Consequently, antifoam 6T80 was chosen for subsequent use and scale-up to bioreactors. This selection was based on its absence of growth inhibitory effects on the test cultures and its low minimum effective concentration required for foam suppression.

3.5. Assessment of the Effect of Antifoam Agents on Cultivation of Bacillus subtilis MGMM38 Strain in Small Parallel Bioreactors

A cultivation experiment of the *B. subtilis* MGMM36 strain was conducted in parallel bioreactors with the addition of vegetable oil and an antifoam agent. As can be seen from the figure, no differences in CFU/ml values were observed throughout the entire fermentation process (Figure 5).

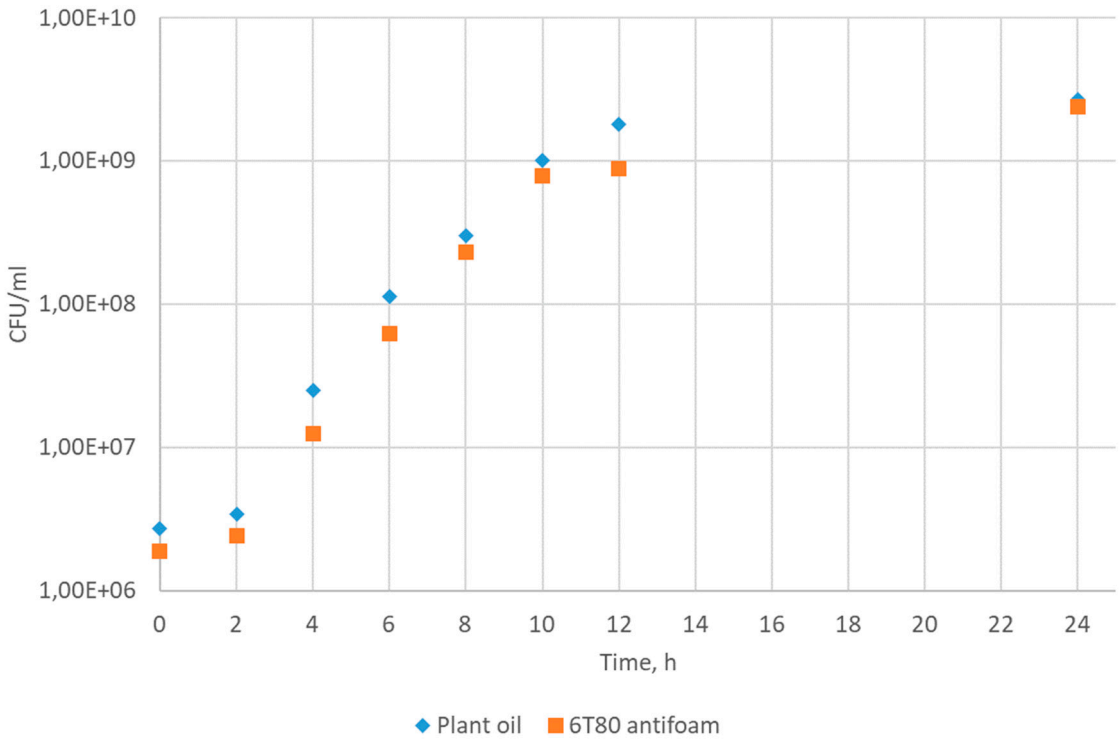

Figure 5. Growth curve of the *B. subtilis* strain in parallel small-volume bioreactors in LB medium. On the X-scale shown absolute amount of the life cells in ml (CFU/ml).

The final CFU titer after 24 h of incubation was the similar under both conditions, at 2.69×10^9 CFU/ml for plant oil, and 2.39×10^9 CFU/ml for 6T80 antifoam agent.

3.6. Assessment of the Antifoam Agent's Impact on the Glycerol Dehydrogenase Biosynthesis in Cells P. putida LN6160 Strain as a Model System

Experiments conducted to assess the impact of the antifoam agent on the protein synthesis process in bacteria using SDS-PAGE electrophoresis showed that the applied antifoam agent 6T80 does not exert an inhibitory or negative effect on the efficiency of target protein synthesis (Figure 6).

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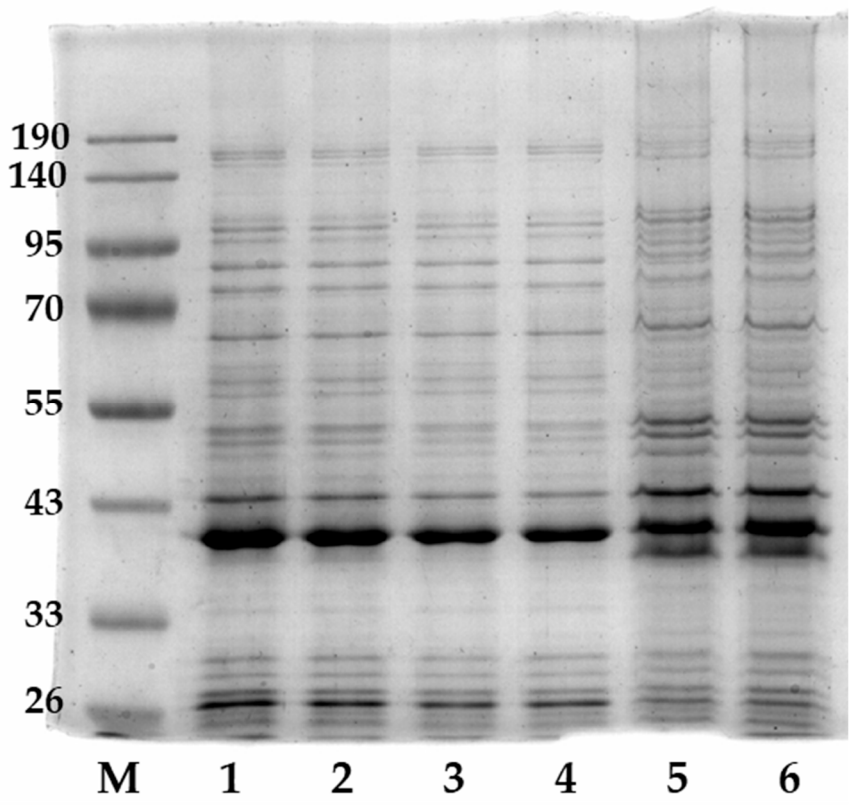

Figure 6. 12% SDS-PAGE gel, staining with Coomasie Brilliant Blue R250. M –Blue Plus® V Protein Marker (10–190 kDa) (TransGen Biotech, Beijing, China), Fractions of *P. putida* LN6160:pJeM2:gld: 1 – *P. putida* LN6160:pJeM2:gld lysate (with plant oil), 2 – *P. putida* LN6160:pJeM2:gld lysate (with antifoam 6T80), 3 – *P. putida* LN6160:pJeM2:gld supernatant (with plant oil), 4 – *P. putida* LN6160:pJeM2:gld supernatant (with antifoam 6T80), 5 – *P. putida* LN6160:pJeM2:gld cell debris (with plant oil), 6 – *P. putida* LN6160:pJeM2:gld cell debris (with antifoam 6T80).

4. Discussion

The selection of an optimal antifoam is a critical step in the development and scale-up of fermentation processes. Despite their widespread use, many industrial antifoams exhibit detrimental side effects, including inhibition of culture growth, induction of cellular stress, and reduction in productivity [9, 28]. Notably, several cases also report a positive effect on the yield of target products, such as recombinant proteins. For instance, in the production of a fluorescent protein in *P. pastoris* cells, the addition of antifoams P2000, SB2121, and J673A doubled the yield of the synthesized protein [1]. This indicates a complex, system-dependent interaction between antifoams and cells and underscores the need for empirical screening and a physicochemical rationale for their action. Consequently, the development of novel formulations that combine high foam-suppressing efficacy with minimal impact on producer physiology remains highly relevant. In the present work, new silicone-polyol emulsions were created and subjected to comprehensive biological testing on a range of taxonomically diverse microorganisms. A key result of this study is the identification of selective antibacterial activity in one of the developed agents (3L10). This formulation exhibited pronounced inhibitory action against Gram-positive test strains (*B. subtilis*, *L. plantarum*, *C. glutamicum*), while showing no significant effect on the growth of Gram-negative bacteria (*E. coli*, *P. fluorescens*). The most sensitive culture was *Corynebacterium glutamicum*, for which growth suppression was observed even at low agent concentrations. This effect is likely due to fundamental differences in cell envelope structure. Gram-negative bacteria are protected by an outer membrane containing lipopolysaccharides, which serves as an effective barrier against many hydrophobic and amphiphilic

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compounds. In contrast, the single cytoplasmic membrane of Gram-positive bacteria is more accessible for interaction with amphiphilic molecules. *C. glutamicum* possesses a distinctive cell wall structure, containing mycolic acids that form an external hydrophobic layer [29]. We hypothesize that the non-ionic amphiphilic component L10, part of agent 3L10, can specifically interact with this lipid layer. This interaction likely disrupts the lipid bilayer's organization, increases its permeability, and ultimately leads to growth inhibition or cell death. This specific selectivity makes agent 3L10 unsuitable for fermentations involving Gram-positive producers. However, this also indicates its potential value as a model compound for studying the mechanisms of interaction of surfactants with bacterial membranes. Although experiments with growth on 96-well plates revealed both the inhibitory and stimulating effects of certain agents (for example, on *E. coli* and *L. plantarum*), this analysis format has significant limitations associated with poor aeration and insufficient mixing of samples. For further investigation, we have determined the minimum effective concentrations (MEC) of defoamers necessary to completely suppress foaming under model conditions. For the most promising formulations, the MEC was approximately 0.067% (by volume). This parameter is key for subsequent process optimization, as it allows for the minimization of both potential toxic effects and negative impacts on oxygen mass transfer. Based on a comprehensive analysis, which considered the absence of inhibition in plate and microplate tests, a low MEC, and emulsion stability, the agent 6T80 was selected for further study. Its neutrality toward cell physiology was confirmed in two model fermentation systems. First, in parallel cultivations of *B. subtilis* in laboratory mini-bioreactors, the dynamics of viable cell accumulation (CFU/ml) when using agent 6T80 showed no statistical difference from the control, where standard vegetable oil served as the defoamer. Second, in a heterologous expression system for glycerol dehydrogenase in *P. putida*, the addition of antifoam 6T80 had no negative effect on the accumulation level of the target recombinant protein, as evidenced by electrophoretic analysis.

5. Conclusions

This study demonstrates that the influence of antifoams on the fermentation process is multifaceted, being determined by both their chemical composition and the biological characteristics of the producer. Our developed approach, which combines the chemical design of emulsions, cytotoxicity screening that accounts for the taxonomic diversity of test cultures, determination of MECs, and validation under conditions close to industrial scale, enables the rational selection of highly efficient and biocompatible formulations. The discovered selective activity of agent 3L10 against Gram-positive bacteria, particularly *C. glutamicum*, warrants further investigation at the molecular level. Nevertheless, it already underscores the necessity for thorough preliminary testing of antifoams for specific producer strains. Agent 6T80, which exhibited no inhibitory properties and demonstrated effectiveness in model fermentations, represents a promising candidate for further trials in targeted biotechnological processes utilizing Gram-negative and, potentially, a range of Gram-positive hosts.

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