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THE EFFECT OF GLUCOSE ON *Streptococcus mutans* ATCC 25175 GROWTH BY VARIATION OF CONCENTRATION AND INCUBATION TIME

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ABSTRACT

Streptococcus mutans is one of the main causative agents of dental caries. Glucose has been reported as one of the factors influencing the growth of *S. mutans*. This study aims to determine the effect of glucose concentration and incubation time on the growth of *S. mutans*. The glucose concentrations used in this study were 0, 2, 4, 6, 8, and 10%. The incubation times used were 1, 10, and 15 hours. The result showed that glucose concentration significantly affects the growth of *S. mutans*. Higher glucose level lead to increase bacterial growth, with the highest absorbance observed at 10% glucose after 10 hours of incubation. Specific growth rate (μ) analysis further supports this finding, showing that *S. mutans* grows more rapidly during the exponential phase (1–10 hours) with increased glucose concentration, while the growth rate stabilizes or declines during the later phase (10–15 hours). This result indicate that both glucose availability and incubation time are critical factors influencing the growth dynamics of *S. mutans*.

Keywords: *Streptococcus mutans,* glucose concentration, incubation time

INTRODUCTION

Dental caries remains one of the most prevalent oral health problems worldwide, primarily caused by the accumulation of bacterial biofilms on tooth surfaces (Syaflida et al., 2023). *Streptococcus mutans* is a key pathogenic bacterium in the development of dental caries due to its ability to metabolize carbohydrates, particularly glucose, into acid through glycolysis (Utamaningyas et al., 2023). This acid production leads to the demineralization of tooth enamel, contributing to cavity formation (Ambarawati et al., 2020).

Glucose plays a crucial role in the growth and metabolic activity of *S. mutans* (Matsumoto-Nakano, 2018). The availability of glucose not only affects the bacterial growth rate but also influences biofilm formation and acid production (Fina Maghfirah, Dewi Saputri, 2017). Different glucose concentrations can lead to varying bacterial growth patterns, which in turn impact cariogenic potential (Alves et al.,

2025). Additionally, incubation time is another critical factor that determines bacterial proliferation and acidogenicity (Widyawati et al., 2022). Understanding the interplay between glucose concentration and incubation time is essential in evaluating the conditions that promote or inhibit *S. mutans* growth (Praptiningsih et al., 2022).

Several studies have examined the effect of carbohydrate sources on *S. mutans* growth; however, limited research has systematically analyzed the combined influence of glucose concentration and incubation time. This study aims to investigate how different glucose concentrations and incubation periods affect the growth of *S. mutans*, providing insights into its metabolic behavior and potential strategies for caries prevention.

By elucidating the impact of glucose on *S. mutans* growth under controlled conditions, this study may contribute to the development of targeted interventions to mitigate dental caries, such as dietary modifications and improved oral hygiene practices. The findings may also provide valuable information for the formulation of preventive dental treatments aimed at disrupting the metabolic pathways of cariogenic bacteria.

METHODS

Materials

The materials and equipment required for this experiment include Mueller-Hinton (MH) broth as the growth medium, prepared with varying glucose concentrations of 0, 2, 4, 6, 8, and 10%. The experiment also requires a spectrophotometer, sterile test tubes, erlenmeyer flasks. Additional equipment includes a 37°C incubator, anaerobic jar, pipettes and micropipettes, autoclaved, distilled water, sterile loops, and laminar flow cabinets. Data recording materials such as notebooks, spreadsheets, or software for statistical analysis are also necessary to process and interpret the results effectively.

Research Path

1. Culture preparation

The bacteria are grown in Mueller-Hinton (MH) broth at 37°C for 18 hours to obtain an active culture. After incubation, the bacterial suspension is standardized to an OD600 of approximately 0.1 to ensure uniform initial bacterial concentration before inoculation into the experimental medium (Hardini Yanis & Putriany Agustin, 2020).

2. Sample preparation with different glucose concentrations

Mueller-Hinton broth prepared with different glucose concentrations (0, 2, 4, 6, 8, and 10%). Each medium is inoculated with

the standardized bacterial culture at 1% v/v to ensure consistent bacterial density across all treatments. A total of 2.4 mL of Mueller Hinton media was added into a microtube, followed by the addition of 0.3 mL of *S. mutans* bacterial culture. The inoculated cultures are then incubated at 37°C under anaerobic conditions to promote bacterial growth (Nguyen et al., 2022).

3. Absorbance measurement

To monitor bacterial growth, optical density (OD600) measurements are taken at specific time intervals (1, 10 and 15 hours) using a spectrophotometer. The absorbance values are recorded for each glucose concentration to assess bacterial proliferation over time (Satari et al., 2019).

4. Calculation of specific growth rate (μ) and growth percentage

The specific growth rate (μ) is determined using the following formula:

$$\mu = \frac{In\ At - In\ A0}{t2 - t1}$$

where At is the absorbance at a specific time, A0 is the absorbance at time 0, and t is the incubation time (in hours). The growth percentage is calculated to compare bacterial growth in different glucose concentrations relative to the control (0% glucose), using the formula:

$$\%\ Growth = \left(\frac{At\ (treatment) - A0}{A0}\right) x100\%$$

where At (treatment) is the absorbance at time t for a given glucose concentration, A0 is the absorbance at time t for the control (0% glucose). Growth percentage values greater than 100% indicate that glucose enhances bacterial growth, while values below 100% suggest growth limitation or inhibition (Dinis et al., 2022).

Data Analysis

The results reported as the mean ± standard deviation. Variations in specific growth were in triplicate. Statistical analysis was conducted using Microsoft Excel with significance set at $P < 0.05$.

RESULTS AND DISCUSSION

The variation in glucose concentrations was used to evaluate the effect of different glucose levels on the growth of *S. mutans*. This range allows the observation of how increasing glucose availability influences bacterial proliferation. Lower concentrations help assess the minimum requirement for growth stimulation, while higher concentrations reveal whether excessive glucose enhances or inhibits bacterial growth. By using multiple concentrations, a clearer understanding of the dose-response relationship between glucose and *S. mutans* growth can be obtained (Zubaidah et al., 2022).

Measuring the OD600 (optical density at 600 nm) before using the bacteria in the experiment is important to determine the bacterial concentration. This ensures that the bacterial culture has reached the desired growth phase, typically the mid-log phase, where the cells are actively dividing and most metabolically active (Situmeang et al., 2022). Standardizing the bacterial density helps ensure consistency and reliability of the experimental results.

Table 1 demonstrate that glucose concentration and incubation time significantly influence the growth of *S. mutans*. At the one hour incubation period, bacterial growth remained relatively low across all glucose concentrations, though a gradual increase was observed as glucose concentration rose. At 0% glucose, no bacterial growth was detected, indicating that glucose serves as a crucial nutrient source for *S. mutans*. The absorbance values increased from 2.396 at 2% glucose to 7.098 at 10%, suggesting that even within a short incubation time, higher glucose levels can stimulate bacterial proliferation (Zhang et al., 2025). However, at 6% and 8% glucose, the growth plateaued slightly (3.811 and 3.804, respectively), possibly due to a temporary adaptation phase or fluctuations in early cell division (Figure 1).

Table 1. Absorbances and % growth of *S. mutans* with incubation times at 1, 10 and 15 hours

Incubation times (hour)	Glucose (%)	Absorbances at λ 619 nm			% Growth			X ±SD
		1	2	3	1	2	3	
1	0	0.069	0.071	0.072	0	0	0	0
	2	0.071	0.073	0.073	2.899	2.899	1.389	2.396 ± 0.872
	4	0.072	0.074	0.073	4.348	4.225	1.389	3.321 ± 1.674
	6	0.074	0.072	0.074	7.246	1.408	2.778	3.811 ± 3.052
	8	0.073	0.074	0.073	5.797	4.225	1.389	3.804 ± 2.234
	10	0.075	0.076	0.076	8.696	7.042	5.556	7.098 ± 1.570
10	0	0.251	0.249	0.252	0	0	0	0
	2	0.377	0.373	0.371	50.199	51.406	47.222	49.609 ± 2.153
	4	0.392	0.397	0.395	56.175	59.438	56.746	57.453 ± 1.742
	6	0.411	0.419	0.414	63.745	68.273	64.286	65.434 ± 2.472

Incubation times (hour)	Glucose (%)	Absorbances at λ 619 nm			% Growth			X ±SD
		1	2	3	1	2	3	
	8	0.456	0.453	0.459	81.673	81.928	82.143	81.914 ± 0.235
	10	0.499	0.491	0.497	98.805	97.189	97.222	97.738 ± 0.923
15	0	0.971	0.956	0.955	0	0	0	0
	2	1.232	1.225	1.223	26.879	28.138	28.063	27.693 ± 0.706
	4	1.339	1.337	1.335	37.899	39.854	39.791	39.181 ± 1.110
	6	1.423	1.420	1.421	46.549	48.536	48.796	47.960 ± 1.229
	8	1.506	1.507	1.505	55.098	57.636	57.592	56.775 ±1.452
	10	1.644	1.642	1.644	69.400	71.757	72.147	71.101 ± 1.486

Table 2. Specific growth rates (μ) of *S. mutans* at different glucose concentrations

Glucose (%)	μ (1h-10h)	μ (10h-15h)
0	0.1407	0.2687
2	0.1825	0.2377
4	0.1875	0.2440
6	0.1925	0.2464
8	0.2031	0.2389
10	0.2088	0.2397

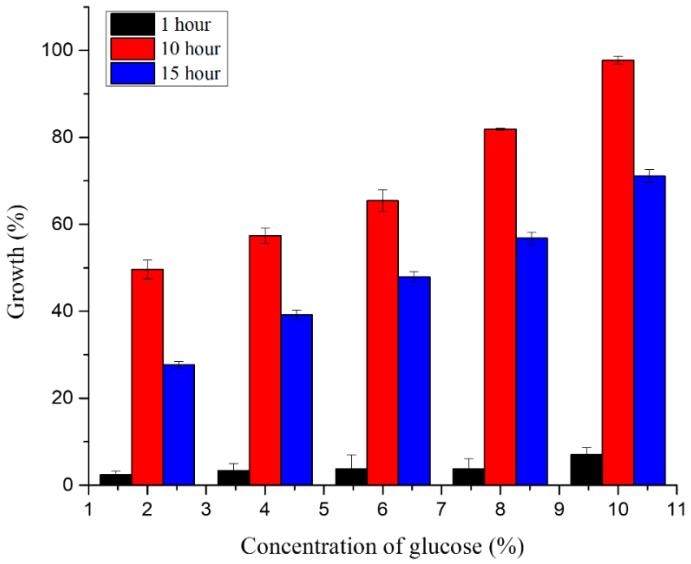

Figure 1. The effect of concentration and incubation times with % growth of *S. mutans*

A more substantial growth was observed after 10 hours of incubation. The absorbance values increased consistently with rising glucose concentrations, with the highest growth recorded at 10% glucose (97.738 ± 0.923). This indicates that *S. mutans* was in its exponential (log) growth phase at this point, actively metabolizing the available glucose to support rapid cell division (Ambarawati et al., 2020). The linear trend from 2% to 10% glucose highlights the importance of glucose availability during this growth phase.

Interestingly, at 15 hours of incubation, the bacterial growth began to decline across all glucose concentrations, despite still being elevated compared to the early time points. While 10% glucose still showed the highest absorbance (71.101 ± 1.486), the value had decreased from that at 10 hours. This decline may indicate the transition of the bacterial culture into the stationary phase or even the onset of the death phase. Factors contributing to this decrease could include nutrient depletion, accumulation of toxic metabolic byproducts such as lactic acid, or a drop in environmental pH, all of which can inhibit further growth (Alves et al., 2025).

In summary, glucose concentration positively correlates with *S. mutans* growth, with 10% glucose supporting the most robust proliferation. However, prolonged incubation beyond 10 hours leads to a decline in growth, likely due to changes in the culture environment. These findings highlight the importance of both nutrient availability and incubation time in understanding bacterial growth dynamics.

The specific growth rate (μ) is a critical parameter used to evaluate the rate at which microorganisms grow within a given time interval. Table 2 showed that μ was calculated across two times intervals: from 1 to 10 hours (μ_{1-10}) and from 10 to 15 hours (μ_{10-15}), to assess the effect of glucose concentration on the growth dynamics of *S. mutans*.

During the 1–10 hours interval, the μ values showed a clear increasing trend with rising glucose concentrations. Starting from 0.1407 at 0% glucose, the growth rate progressively increased, reaching 0.2088 at 10% glucose. This indicates that glucose availability plays a major role in accelerating the growth of *S. mutans* during the exponential phase. As a primary energy source, glucose supports cellular metabolism and division, resulting in a faster overall growth rate during this period.

In contrast, during the 10–15 hours interval, the growth rate pattern changed. The μ values remained relatively stable across glucose concentrations ranging from 2% to 10%, fluctuating within a narrow range of

0.2377 to 0.2464. Interestingly, the highest μ value (0.2687) was observed at 0% glucose, which is unexpected. This anomaly may reflect changes in environmental conditions such as nutrient depletion, acid accumulation, or stress from high cell density that could inhibit further growth regardless of glucose availability. It is possible that cells were entering the stationary phase, where metabolic activity and cell division begin to slow down.

Overall, these findings suggest that glucose significantly enhances the growth rate of *S. mutans* during the exponential phase (1–10 hours), but its influence diminishes in the later stage of growth (10–15 hours), where environmental stress and other limiting factors begin to dominate. This highlights the complex interplay between nutrient availability and culture conditions in regulating bacterial growth dynamics.

CONCLUSIONS

The study demonstrates that glucose concentration significantly affects the growth of *S. mutans*. Higher glucose levels lead to increased bacterial growth, with the highest absorbance observed at 10% glucose after 10 hours of incubation. Specific growth rate (μ) analysis further supports these findings, showing that *S. mutans* grows more rapidly during the exponential phase (1–10 hours) with increasing glucose concentration, while the growth rate stabilizes or declines during the later phase (10–15 hours). These results indicate that both glucose availability and incubation time are critical factors influencing the growth dynamics of *S. mutans*. Glucose, at both low and high concentrations, affects the growth of *S. mutans* on teeth.

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