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Research Article

Study the Effect of Glycerol on Shape, Size, and Growth Rate of *Escherichia Coli*

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Keywords

E. Coli,
Glycerol,
Growth rate.

Abstract

Carbohydrate is a decisive factor in the growth of bacteria. Glucose is the most common carbon source of bacterial growth. However, the role of glycerol on bacterial growth remained to be clear. In this paper, the effects of glycerol on HF19 and MC1061 growth was examined. The optical density and colony formation unit per milliliter of cultured media was measured. Moreover, the cell dry mass of the cultured media has been assessed. In order to directly study the effect of glycerol on the E. coli cell proliferation, hemocytometer was used. The strong correlation was found between optical density and CFU/ml in the all incubation period in the control groups. The data showed very weak correlation between optical density and CFU/ml in the glycerol-treated group after 24-hour incubation. The data also indicated that glycerol increased the optical density, colony formation unit and cell dry mass of the sample. Although the data indicated strong relation in the control groups, in all incubating period, in the glycerol-treated group, we observed strong relation only in 6-hour and 12-hour incubation period. The data suggests that in long time glycerol incubation, glycerol induced changes in shape and/or size of E. coli in both strains.

1. Introduction

Escherichia coli (E. coli) as a Gram-negative, rod-shaped, facultative anaerobic bacterium described by Theodor Escherich in 1885[1]. This bacterium is commonly present in the intestines of humans and animals and is part of the healthful bacterial flora in the human gut[2]. Although most strains of this bacterium like HF19 and MC1061 harmlessly colonize the gastrointestinal tract of humans and animals as a normal flora [3], some strains like Shigatoxigenic Escherichia coli (STEC) [4] and verotoxigenic E. coli (VTEC) [5] are pathogenic and lead to diarrhea [6], abdominal pain [7], fever [8], and sometimes vomiting [9]. Physical and nutritional parameters play important roles in the generation of the required energy and cellular biosynthesis of E. coli. Among all physical parameters, osmotic pressure [10], pH [11], temperature [12], hydrostatic pressure [13], and the amount of moisture in the medium where the organism is growing [14], are more important. The nutritional factors are accessed as the amount of carbon [15], nitrogen [16], phosphorous [17], and other required trace elements in the growth medium. E. coli is capable of consuming different compounds as the source of carbon, such as glycerol and glucose [18]. Carbon source addition to the nutrient broth may increase both the overall growth rates and biomass of bacteria over time. The effect of nutrition on the bacteria growth could be beneficial for lab purposes, since less time needed to grow up bacteria.

2. Discussion

In this study, our hypothesis is that glycerol affects the shape and size of cells, which in turn alter the scattering of light and hence OD. Therefore, the goal of this study is to compare the optical and cell density of the cell suspension

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grown in the presence and absence of glycerol. To this aim, we measured the OD, CFU/ml and cell dry mass.

2.1.Material and Method

The procedure is as follow:
Two strains of E. coli (HF19 and MC1061) were cultured on the solid media (Lysogeny broth) for 14-16 hours and then a single colony was incubated in the liquid culture for 6, 12 and 24 hours (using shaker in 37°C).
Measure the OD595 of each culture.

We diluted the cultures serially and plated them on the agar plate to measure the CFU/ml.

Measuring the cell dry mass.

In order to asses our hypothesis, we needed to have two sets of liquid culture, medium with and without glycerol. For each strain, we had two groups, control and treated. In the treated group 10 mL LB and 250 µL (25% of LB) glycerol (according to the literature, this concentration is the best for growing bacteria by adding to the media) was added. Control group, on the other hand, received 10 mL LB and 250 µL (25% of LB) water in the culture media. After preparing the liquid media, we put the single colony of each strain in each flask and shacked them for 6, 12 and 24 hours. The results for strain HF19 are shown on Figure 1.

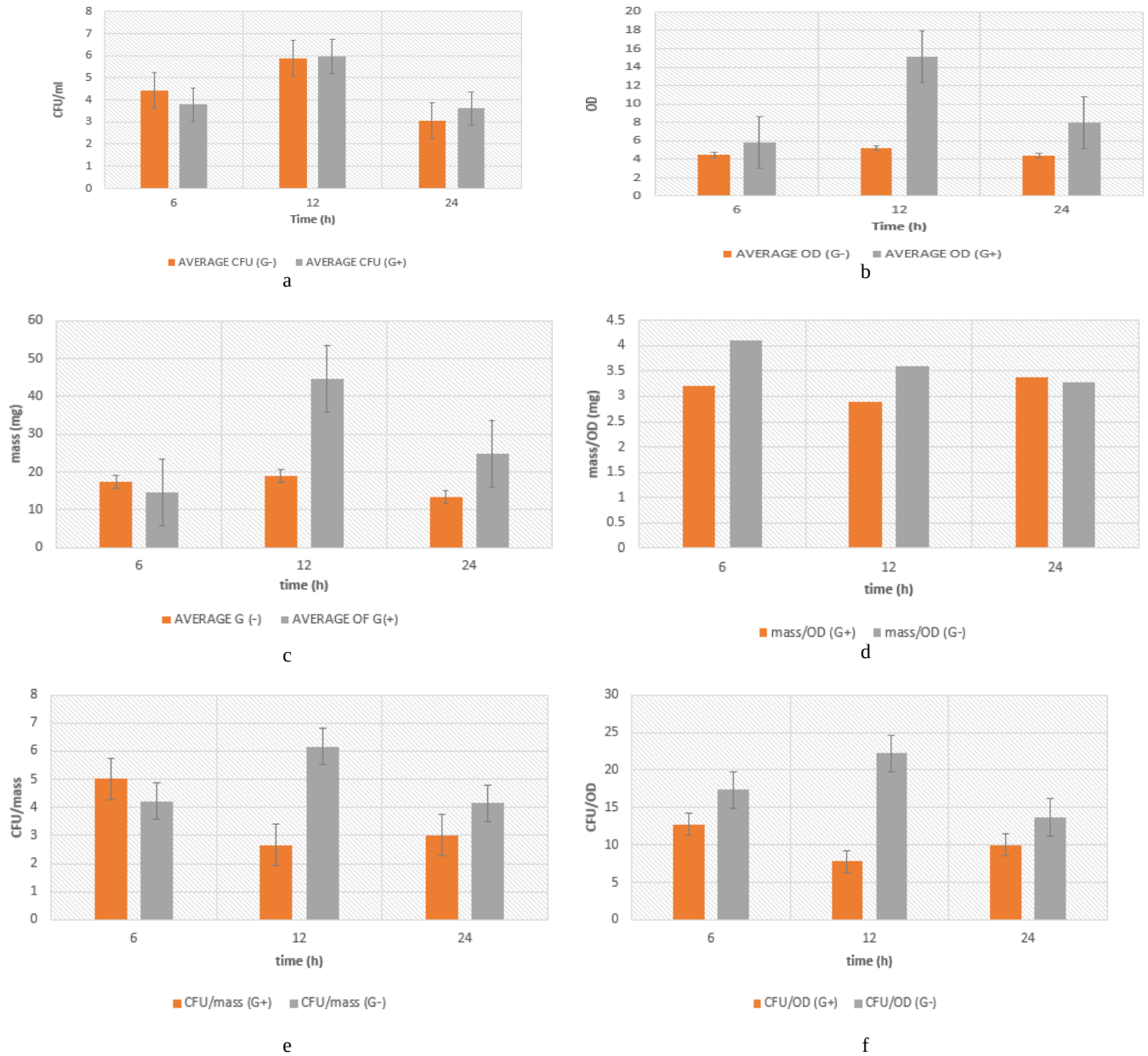

Figure 1. The effect of glycerol on CFU, OD and mass on HF19

Next, we did the same process for MC106. The results shown below in Figure 2.

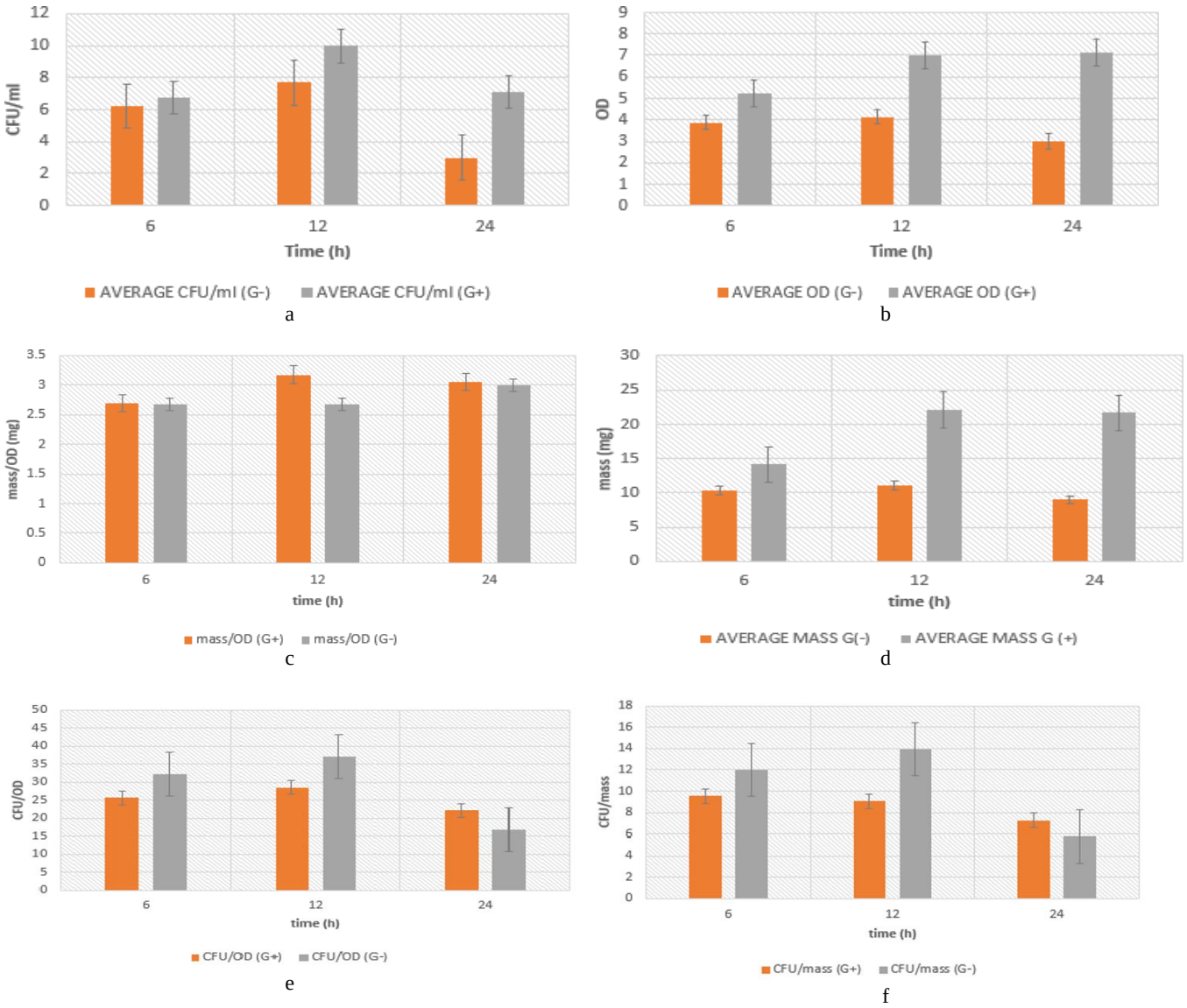

Figure 2. The effect of glycerol on CFU, OD and mass on MC1061

To determine the effects of the number of cells on OD, we used hemocytometer. Hemocytometer enables us to measure the concentration of cells in our liquid culture. The results related to the HF19 after 24 hours incubation are shown in Figure 3.

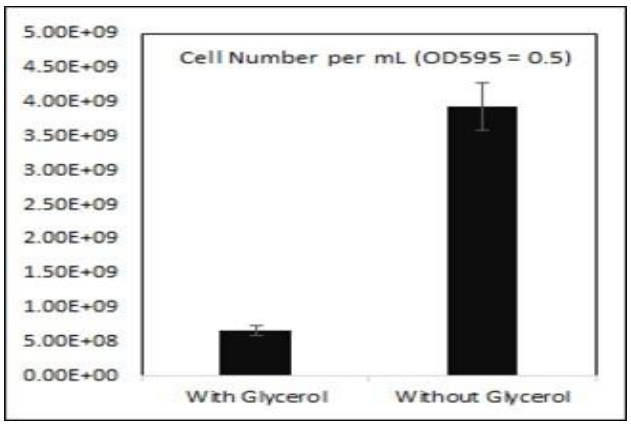

Figure 3. The results related to the HF19 after 24 hours incubation

To assess the bacterial growth population, we incubated the viable cells of the bacterium into the sterile broth under optimal growth conditions. Since the bacterium utilizes the components of the media, we expected to have bigger cell and higher cellular mass. We studied the dynamics of the bacterial growth by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time. Therefore, the curve is a sigmoid curve, which is known as standard growth curve. For obtaining the growth curve of the HF19 and MC1061 cells, we assessed the cell growth by using OD. The initiate OD for these two strains was 0.01. The growth curve for these strains are shown in Figures 4, 5.

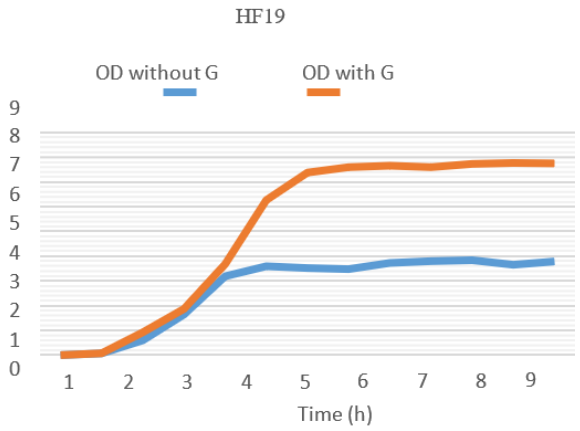

Figure 4. Growth curve of HF19

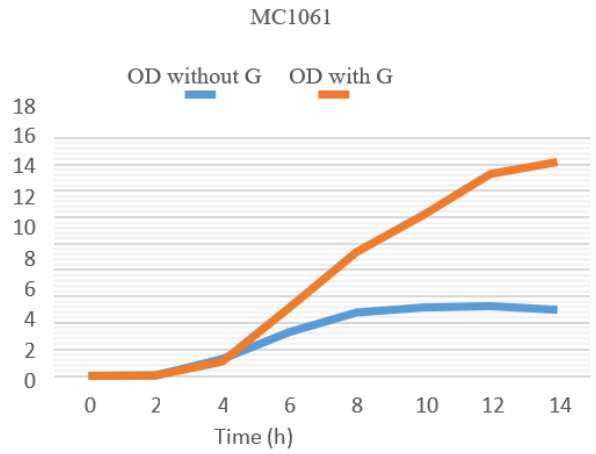

Figure 5. Growth curve of MC1061

4. Conclusion

There is a significant difference between OD of cells in the presence and absence of glycerol especially after 12 hours incubation for HF19, and 24 hours for MC1061. In addition, CFU/ml was change during the experiment; however, we did not observe a good correlation between CFU/ml and OD change. We observed a good correlation between the cell mass and OD change; however. The results could be because of the cells death or, the different weight per cell in the absence and presence of glycerol. To know about the correlation between OD and number of cells, we used hemocytometer. By using it, we could not see a good correlation between these two factors and it can confirm our hypothesis that glycerol can change the size or/and shape of the cells by interring to the cells.

Conflict of Interest Statement

There is not any conflict of interest in this paper.

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