Polyethylene glycol exerted toxicity to growth of *Bacillus subtilis* NRS-762

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Abstract

Polyethylene glycol is commonly used in fermentations as an anti-foam for preventing the rise of foam to the top plate of the bioreactor, which increases contamination risk. However, its potential toxicity to growth of various microorganisms is not well understood at the strain and species level. Hence, the objective of this study was to understand the impact of different concentrations of polyethylene glycol (0, 1, 5 and 10 g/L) on the aerobic growth of *Bacillus subtilis* NRS-762 (ATCC 8473) in LB Lennox medium in shake flasks at 30 ^{o}C and 230 rpm rotational shaking. Experiment results indicated that polyethylene glycol (PEG) (molecular weight ~8000 Da), at all concentrations tested, exerted some toxicity towards the growth of *B. subtilis* NRS-762 in LB Lennox medium. Specifically, maximal optical density obtained declined with greater exposure to PEG in a concentration-dependent manner, up to a threshold concentration of 5 g/L PEG. For example, maximal optical density obtained in *B. subtilis* NRS-762 without addition of PEG was 4.4, but the value obtained on exposure to 1 g/L of the anti-foam decreased to 4.1 and a further 3.8 on exposure of cells to 5 g/L and 10 g/L PEG. Similarly, growth rates of *B. subtilis* NRS-762 also decreased in a concentration-dependent manner with PEG concentration up to a threshold concentration of 5 g/L PEG. pH variation in culture broth, however, revealed that the pH profiles for exposure to PEG at all concentrations overlapped each other and was similar to the one of cells without exposure to the anti-foam; thereby, highlighting that metabolic processes in *B. subtilis* NRS-762 were not significantly affected by exposure to PEG. Collectively, polyethylene glycol anti-foam exerted toxicity effect on *B. subtilis* NRS-762 biomass formation, and possibly metabolism. The latter may not be sufficiently significant to affect the types of metabolites secreted by the bacterium, and thus, could not be detected by measurement of culture broth pH. Overall, the results should inform the choice and concentration of PEG for culturing *B. subtilis* in biotechnological applications.

Keywords: dose response, polyethylene glycol, toxicity, biomass formation, metabolism, optical density, pH variation, culture broth, *Bacillus subtilis*, growth rate,

Subject areas: biotechnology, microbiology, biochemistry, cell biology, bioengineering,

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Highlights

1) Polyethylene glycol (PEG, molecular weight ~8000 Da) exerted toxicity effects on *Bacillus subtilis* NRS-762 (ATCC 8473) during growth in LB Lennox medium at 30 °C, by reducing biomass formation and growth rates in a concentration-dependent manner.

2) Specifically, optical density of *B. subtilis* NRS-762 culture was reduced from 4.4 to 4.1 upon addition of 1 g/L PEG, which dropped to 3.8 on addition of 5 and 10 g/L PEG.

3) However, pH profiles of *B. subtilis* NRS-762 overlapped each other irrespective of the concentrations of PEG used; thereby, suggesting that PEG exerted its toxicity effects through affecting processes related to cell division and biomass formation, and not metabolites secreted.

Introduction

Polyethylene glycol is commonly used as an anti-foam in microbial fermentation, especially in bioreactors, where profusion of dissolved oxygen and impellar stirring combined to result in rising of foam to the top plate of the bioreactor which increases the risk of contamination. As an additive to microbial fermentation, bacteria capable of metabolizing polyethylene glycol has been reported.[1 2 3] On the other hand, studies have also investigated the effect of polyethylene glycol on the viability of spores.[4] More importantly, polyethylene glycol has been shown to induce the transformation of *Bacillus subtilis* cells by chromosomal and plasmid DNA.[5 6] Additionally, polyethylene glycol is also capable of inducing the fusion of protoplasts of *B. subtilis*.[7 8] However, relatively little is known about the effect of polyethylene glycol on the growth of *B. subtilis*, which is commonly used in biotechnology for expressing recombinant proteins in bioreactor based production of useful pharmaceuticals and value-added products.[9 10 11]

Thus, this study aims to understand the effect of different concentrations of polyethylene glycol (0, 1, 5, and 10 g/L, average molecular weight 8000 Da) on the aerobic growth of *Bacillus subtilis* NRS-762 (ATCC 8473) in LB Lennox medium contained in shake flasks. Specifically, optical density and pH would be measured to ascertain the time course impact of exposure to different concentrations of polyethylene glycol on bacterial growth and metabolism. pH was used as a proxy parameter for the net amount of metabolites secreted into the culture broth during growth.

Materials and methods

Materials

LB Lennox was purchased from Difco and used as is. Composition of the medium was in [g/L]: Tryptone, 10.0; Yeast extract, 5.0; NaCl, 5.0. Polyethylene glycol of molecular weight ~8000 Da was purchased from Sigma-Aldrich. It was dissolved in deionized water and filtered sterilized with a 0.22 µm Pall polyethersulfone membrane filter during addition to sterilized LB Lennox medium.

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Growth of bacteria in liquid medium

Stock cultures of *B. subtilis* NRS-762 were prepared in 40% glycerol and stored at – 70 ^{o}C prior to use. Glycerol stock cultures were used to inoculate seed cultures of 100 mL LB Lennox medium in 250 mL glass shake flask under aerobic culture conditions at 30 ^{o}C and 230 rpm rotational shaking in a temperature controlled incubator shaker. After 24 hours of incubation, 1 mL of inoculum from the seed cultures was used in inoculating the experiment cultures. Three biological replicates were performed.

Optical density and pH measurement

At appropriate time points, 5 mL of culture broth was obtained for measuring optical density and pH. Optical density was measured at 600 nm using a Shimadzu Biospec Mini spectrophotometer with a quartz cuvette of 10 mm pathlength (volume: 3.5 ml). Appropriate dilution with deionized water was used if the optical density exceeded 1. pH was measured, without any sample dilution, using an Orion 9156 BNWP pH probe fitted to a Mettler Toledo Delta 320 pH meter.

Results and Discussion

Growth of *B. subtilis* NRS-762 in LB Lennox medium under different concentrations of polyethylene glycol revealed some toxicity effect of the anti-foam on growth of the bacterium (Figure 1a). Specifically, without addition of polyethylene glycol, the maximal optical density obtained was 4.4. This value decreased to 4.1 after addition of 1 g/L of polyethylene glycol. Upon addition of 5 g/L and 10 g/L polyethylene glycol, the maximal optical density attained decreased to 3.8, which highlighted that polyethylene glycol exerted negative impacts on growth of *B. subtilis* NRS-762. Besides reduction in maximal optical density obtained, growth was also slower with addition of polyethylene glycol and the effect was concentration-dependent, where higher concentration of polyethylene glycol resulted in slower growth. However, a threshold existed at 5 g/L of polyethylene glycol where there was no further concentration-dependent effect on growth rates and maximal optical density obtained. Specifically, both 5 g/L and 10 g/L polyethylene glycol depressed growth rate and maximal optical density in the same way.

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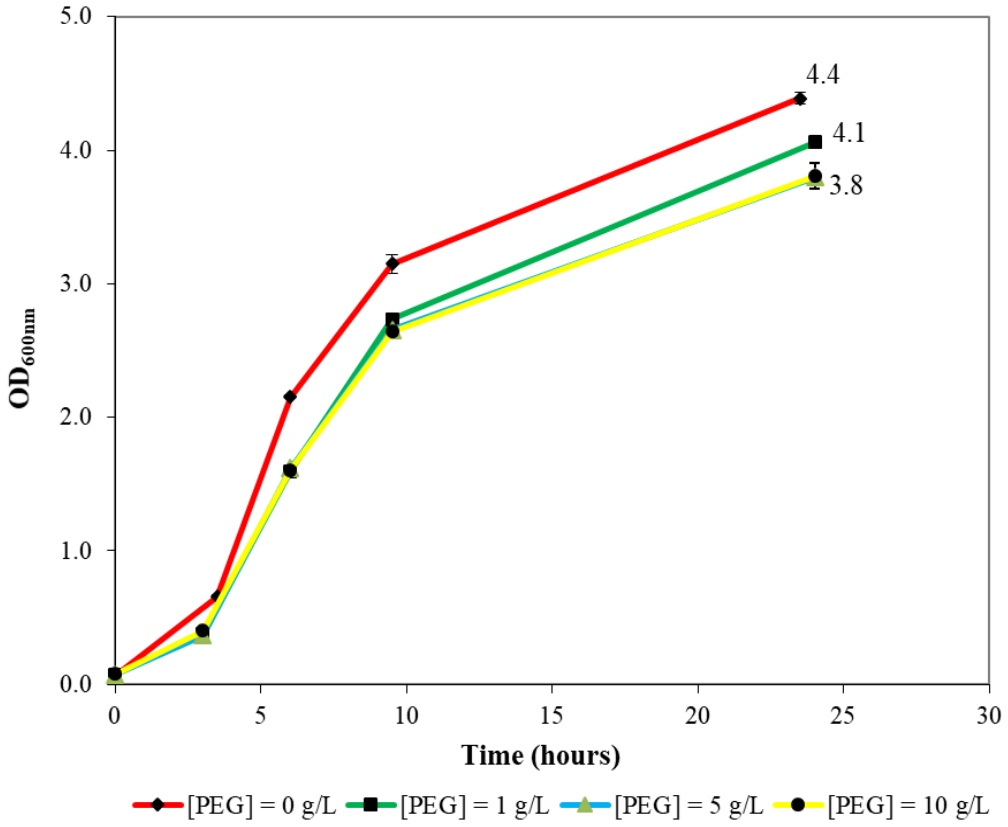

Figure 1a: Aerobic growth of *B. subtilis* NRS-762 in LB Lennox medium at 30 ^{o}C under varying concentrations of polyethylene glycol. Note that the maximal optical density obtained decreased in a concentration-dependent manner with respect to polyethylene glycol.

pH variation with culture time in *B. subtilis* NRS-762 cultures exposed to different concentrations of polyethylene glycol revealed that there was no concentration-dependent impact on pH profile (Figure 1b). Specifically, the same pH profile was obtained independent of the concentration of polyethylene glycol added; thereby, highlighting that the anti-foam has minimal impact on the metabolism of the cells. Thus, polyethylene glycol likely exerted its toxicity to *B. subtilis* NRS-762 through reducing the biomass formation capacity of the bacterium.

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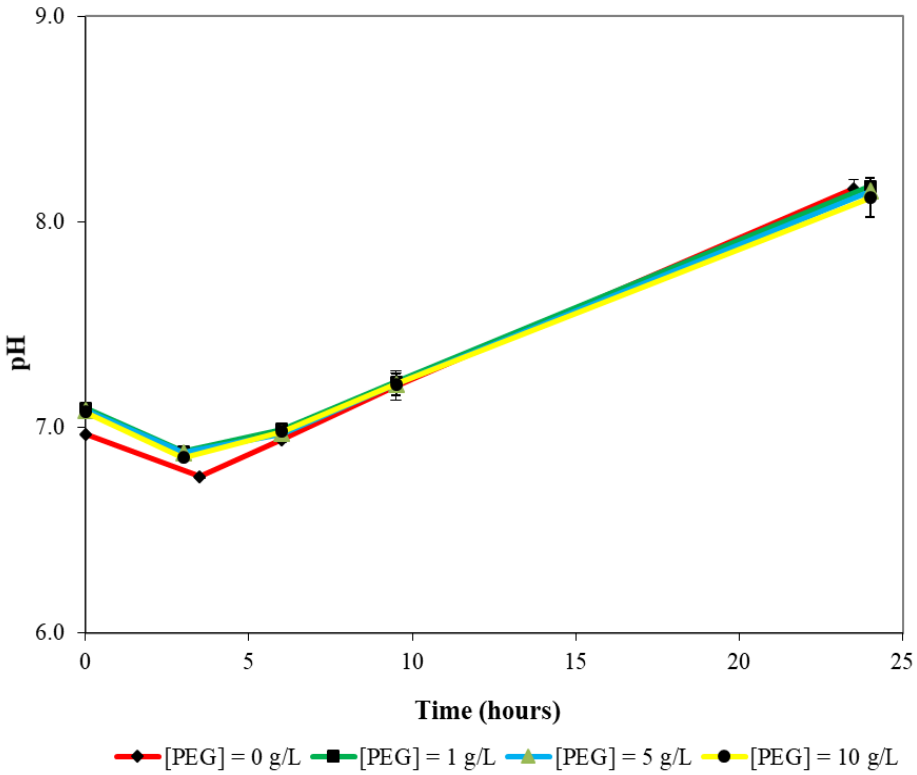

Figure 1b: Variation of pH during growth of *B. subtilis* NRS-762 in LB Lennox medium at 30 ^{o}C under different concentrations of polyethylene glycol. In general, the same trend holds independent of the concentration of polyethylene glycol used; thereby, suggesting that the anti-foam has minimal impact on the metabolism of *B. subtilis* NRS-762. Thus, polyethylene glycol exerted its toxicity effect primarily through reducing the biomass formation capacity of cells.

Conclusions

Polyethylene glycol reduced the maximal optical density of *B. subtilis* NRS-762 in a concentration-dependent manner during aerobic growth of the bacterium in LB Lennox medium at 30 ^{o}C and 230 rpm rotational shaking. Specifically, maximal optical density obtained decreased from 4.4 where there was no polyethylene glycol addition to 4.1 with 1 g/L of polyethylene glycol added. Optical density of 3.8 was obtained when 5 g/L and 10 g/L of polyethylene glycol was added into LB Lennox medium for culturing of *B. subtilis* NRS-762. Growth rates were also slower with addition of polyethylene glycol into the growth medium in a concentration-dependent manner. However, toxicity impact of polyethylene glycol did not manifest as changes to the pH profile obtained during growth of *B. subtilis* NRS-762 under differing concentrations of polyethylene glycol, which suggested that metabolism of cells were not significantly affected by the anti-foam. More importantly, decline in optical density with increasing exposure to polyethylene glycol likely came from effect of the anti-foam on biomass formation processes.

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Conflicts of interest

The author declares no conflicts of interest.

Funding

The author thank the National University of Singapore for financial support.