

Development of a semi-defined medium for high cell density cultivation of *Escherichia coli* in shake flasks: Part 1

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Conflict of Interest

The authors declare no conflict of interest.

Author's contributions

Wenfa Ng conceived the idea, designed and performed the experiments, analyzed the data, and wrote the abstract. Yen-Peng Ting mentored Wenfa Ng, as well as discussed and analyzed the experimental data.

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Comment

An oral presentation describing this work was delivered at the 17th Regional Symposium on Chemical Engineering, 22-23 November 2010, Bangkok, Thailand. The abstract reported in this preprint is an improved version of the one published in the hardcopy conference proceedings. Part 2 of this work describes improvements in medium performance - specifically, higher cell yield as well as a shorter diauxic lag phase and total culture period - achieved through a small reduction in D-Glucose and NH₄Cl concentrations in the medium composition.

Abstract

Sufficient quantities of cells of consistent characteristics are needed for studying biological processes (at the population level) in many areas of applied microbiology research. However, generating the requisite biomass amount by cell culture is usually the rate-limiting step of the experiment given the relatively low biomass yield of many commercial culture media in shake flasks. This work reports the formulation of a semi-defined medium that enabled aerobic high cell density cultivation of *Escherichia coli* DH5 α (ATCC 53868) in shake flasks. The formulated medium (FM) comprises: a buffer system (K₂HPO₄: 12.54 g/L and KH₂PO₄: 2.31 g/L); vitamins and trace elements (yeast extract: 12.0 g/L); salts (NaCl: 5.0 g/L and MgSO₄: 0.24 g/L); and carbon and nitrogen sources (D-Glucose: 6.0 g/L and NH₄Cl: 1.5 g/L). Notable characteristics of this medium are: high buffer capacity (89 mM phosphate), 1:1 molar ratio between D-Glucose and NH₄Cl, and yeast extract providing trace elements and a secondary source of carbon and nitrogen. Preliminary data revealed that an OD_{600nm} of 9 was attained after 24 hours of cultivation at 37 °C, with glucose and NH₄Cl as the main nutritional sources. At 48 hours, the OD_{600nm} reached a maximal value of 11 with yeast extract providing the necessary nutrients for cell growth and biomass formation. The broth's pH varied between 5.5 and 7.8 during cultivation. For comparison, the maximum OD_{600nm} of *E. coli* grown in three commonly used complex media: Nutrient Broth, LB Lennox, and Tryptic Soy Broth (TSB) were 1.4, 3.2 and 9.2, respectively, under identical culture conditions. Finally, FM maintained the viability of a larger population of cells for three days - compared to a population collapse observed in TSB after one day. Collectively, the present findings suggest that the formulated medium may find use as a high cell density aerobic growth medium for *E. coli* in shake flasks. Part 2 of this work describes improvements in medium performance - specifically, higher cell yield as well as a shorter diauxic lag phase and total culture period – achieved through a small reduction in D-Glucose and NH₄Cl concentrations in the medium composition. A preprint of the work is available at <https://peerj.com/manuscripts/6757/>.

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