Development of a semi-defined medium for high cell density cultivation of *Escherichia coli* in shake flask culture system: 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 W. Ng in the research, analyzed the data, and wrote the abstract.

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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 NH_4Cl concentrations in the medium composition.

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Abstract

Sufficient quantities of cells of consistent characteristics are needed for studying processes - at the population level and beyond - in many areas of applied microbiology research. Nevertheless, given the relatively low biomass yield of many commercial culture media in shake flasks, producing the requisite biomass by cell culture is generally the rate-limiting step. This work reports the formulation of a semi-defined medium that enabled aerobic high cell density cultivation of *Escherichia coli* DH5α (ATCC 53868) in shake flasks. The formulated medium (FM) comprises: a buffer system (K_2HPO_4: 12.54 g/L and KH_2PO_4: 2.31 g/L); vitamins and trace elements (yeast extract: 12.0 g/L); salts (NaCl: 5.0 g/L and $MgSO_4$: 0.24 g/L); and carbon and nitrogen sources (D-Glucose: 6.0 g/L and NH_4Cl: 1.5 g/L). Notable characteristics of this medium are: high buffer capacity (89 mM phosphate), 1:1 molar ratio between D-Glucose and NH_4Cl, 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 – most probably fuelled by glucose and NH_4Cl. 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 for three days - compared to a population collapse observed in TSB after one day. Taken together, the present findings suggest that the formulated medium may find use as a high cell density aerobic growth medium for *E. coli* in shake flask. 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 NH_4Cl concentrations in the medium composition. A preprint of the work is available at https://peerj.com/preprints/117v1.

Keywords: high cell concentration, shake-flask, culture medium, aerobic, Gram-negative, optical density, growth medium

Subject areas: Environmental Sciences; Microbiology; Biotechnology; Biochemistry

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