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

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New in this version

Language and logic flow is improved in this version.

Conflicts of interest

The authors declare no conflicts 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 experiment data.

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Abstract

Sufficient quantities of cells of consistent characteristics are needed for studying biological processes (at the population level) in many areas of applied microbiology. However, generating the requisite biomass by cell culture is usually the rate-limiting step of a project 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* 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 ^{o}C, with glucose and NH_4Cl as the main nutrients. At 48 hours, the OD_{600nm} reached a maximum 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 suggested that the formulated medium might 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 NH_4Cl concentrations in the medium composition. An abstract preprint of Part 2 is available at https://peerj.com/preprints/117/

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

Subject areas: Environmental Sciences; Microbiology; Biotechnology; Biochemistry;

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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