# Development of a semi-defined medium for high cell density cultivation of *Escherichia coli* in shake flasks: Part 2

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## New in this version

Sentence structure, punctuation and logic flow was updated in this version.

## Conflicts of interest

The authors declare no conflicts of interest.

## Authors' 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 data.

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**Abstract**

The characteristics of the culture vessel determine, to a large extent, the type of growth medium suitable for use. For example, most growth media for high cell density cultivation are designed for expensive bioreactors operating either in continuous or fed-batch mode, where provision of additional nutrients and/or removal of metabolic waste products from the basal medium (comprising salts, buffer components and small amount of carbon and nitrogen sources) help increase biomass yield by maintaining culture conditions within the range conducive for growth. The inexpensive and ubiquitous shake flask, in contrast, is usually operated in batch mode and contains, at the outset, a comprehensive medium with all necessary nutrients for conversion into biomass, and also serves as a repository for secreted metabolites - some of which are detrimental for cell growth. Thus, designing medium for high cell density cultivation in shake flask is an optimization process with the aim to increase biomass formation while reducing toxic metabolite secretion. This preprint reports improvements to a previously reported semi-defined medium for high cell density aerobic cultivation of *Escherichia coli* DH5α (ATCC 53868) in shake flasks. Specifically, by reducing the concentrations of glucose (from 6.0 to 4.0 g/L) and ammonium chloride (from 1.5 to 1.0 g/L), the following improvements were obtained: a shorter diauxic lag phase (3 versus 5 hours); a higher maximum optical density (12.0 versus 11.0) in a shorter total culture period (27 versus 48 hours), and smaller pH variation during cultivation (6.0 to 7.6 versus 5.5 to 7.8). Similar to the earlier study, glucose and yeast extract served as principal carbon sources in separate growth phases for *E. coli* in the improved formulated medium ($FM_{improved}$). Specifically, an $OD_{600nm}$ of 6.6 was attained after 9 hours of growth on glucose at 37 $^{o}$C. After a lag phase of 3 hours, growth resumed on yeast extract and the $OD_{600nm}$ reached 12.0 after 27 hours. The broth's pH decreased from 7.1 to 6.0 during the first growth phase, whereupon it gradually rose to 7.6 at the end of culture. A smaller pH decrease along with higher biomass yield in the first growth phase suggested that the lower glucose concentration in $FM_{improved}$ might have prevented overflow metabolism (and associated negative effects on growth); thus, resulting in a shorter diauxic lag phase and total culture period. Collectively, increase in cell yield, as well as decrease in total culture time and a shorter diauxic lag phase arise from a small reduction in glucose concentration - which suggested that an optimum exist, beyond which occurrence of overflow metabolism would reduce cell yield and biomass formation.

***Keywords:*** high cell concentration; conical flask; medium formulation; culture medium; medium design; Gram-negative bacteria; Erlenmeyer flask; cell yield; cell physiology; ionic strength;

***Subject Areas:*** Microbiology; Biotechnology; Biochemistry; Environmental Sciences; Bioengineering;



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