

Topic Introduction

Chemostat Culture for Yeast Physiology and Experimental Evolution

Maitreya J. Dunham,¹ Emily O. Kerr, Aaron W. Miller, and Celia Payen

Department of Genome Sciences, University of Washington, Seattle, Washington 98195

Continuous culture provides many benefits over the classical batch style of growing yeast cells. Steady-state cultures allow for precise control of growth rate and environment. Cultures can be propagated for weeks or months in these controlled environments, which is important for the study of experimental evolution. Despite these advantages, chemostats have not become a highly used system, in large part because of their historical impracticalities, including low throughput, large footprint, systematic complexity, commercial unavailability, high cost, and insufficient protocol availability. However, we have developed methods for building a relatively simple, low-cost, small footprint array of chemostats that can be run in multiples of 32. This “ministat array” can be applied to problems in yeast physiology and experimental evolution.



BATCH CULTURE

The most common method of growing yeast cell cultures is the standard “batch” culture. In this regime, a small number of cells are inoculated into nutrient-rich medium and allowed to divide at maximal growth rate through nutrient exhaustion and into saturation. Sampling of the culture for the experiment of choice is most typically done from the saturated culture or from cells growing in mid-log phase before the diauxic shift. Batch culture provides a number of obvious benefits—it is easy, uses glassware commonly available in any laboratory, and is consistent with a vast literature. However, batch cultures can be problematic for certain applications. When comparing cells of different genotypes, for example, differences in the maximal growth rate are a common phenotype. Many other phenotypes co-vary with growth rate, leading to a nonspecific suite of cell biological, gene expression, and other physiological changes that may not be directly related to the mutation of interest (Regenberg et al. 2006; Castrillo et al. 2007; Brauer et al. 2008).

Batch culture has also been a standard for long-term evolution experiments. Depending on the question being asked, batch culture with serial dilution can be a simple and scalable solution (e.g., Zeyl et al. 2003; Lang et al. 2011). However, such cultures are only rarely propagated in a relatively constant environment and growth rate regime because doing so can require a heroic sampling regimen where back dilution is performed multiple times per day (e.g., Torres et al. 2010). More typically, cultures are allowed to exhaust nutrients before transfer to new medium, leading to a variation in growth rate and nutrient access over the evolutionary time course. These discontinuities in selective pressure can lead to complex subpopulation structures in which different genotypes specialize for the various stages of the growth cycle (e.g., dividing a few extra times or failing to die after saturation, shortening lag phase,

¹Correspondence: maitreya@uw.edu

or increasing maximal growth rate, all of which have been observed in bacterial serial-dilution-based evolution experiments [Lenski et al. 1998; Rozen and Lenski 2000]).

CONTINUOUS CULTURE

Cell physiology and experimental evolution can be studied in a more controlled manner using continuous cultures. Continuous culture refers to the utilization of a class of growth apparatus that maintains a constant environment in one of several dimensions, including chemostats (constant growth rate), turbidostats (constant turbidity), and other devices that feed back on pH, oxygen tension, or other parameters. For the purposes of this introduction, we will focus on chemostats. In chemostat culture, cells are grown at a set dilution rate to a nutrient-limited steady state, at which all inputs equal all outputs. This steady state is highly desirable for modeling applications (e.g., Knijnenburg et al. 2009; Reznik et al. 2013) and for perturbation experiments (e.g., Ronen 2006; McIsaac et al. 2012). The precise control over growth rate also allows matching of growth rates between wild-type cultures and more slowly growing mutants, or among diverse strain backgrounds, by forcing the cells to grow at a steady state that is below the maximum growth rate of both (e.g., Hayes et al. 2002; Torres et al. 2007; Skelly et al. 2013). Even small differences in competitive fitness can be accurately measured using the chemostat, making it a useful tool for characterizing individual mutants (Baganz et al. 1998) and collections of strains (Delneri et al. 2008).

Steady state is typically achieved within the first several volume replacements after inoculation of the chemostat and onset of media flow. Steady state can be maintained for up to at least 35 generations before being detectably perturbed by the inevitable influx of *de novo* mutations and subsequent selection, turning a physiology experiment into an evolutionary one. For evolution experiments, the advantages are continuous growth in a stable environment. This assumption is never strictly true, however, as cultures can change in density and media composition as their genotype composition shifts. Despite this complexity, the primary selection pressure is largely maintained by the nutrient limitation, which is selected by the experimenter.

CHEMOSTAT DESIGN

The basic plumbing of all chemostat platforms is generally the same (see Fig. 1). Medium is added at a defined rate while spent medium, including cells, overflows the culture at that same rate. Aeration, if desired, is provided by vigorous stirring and/or bubbling of gas through the culture. The media feed,

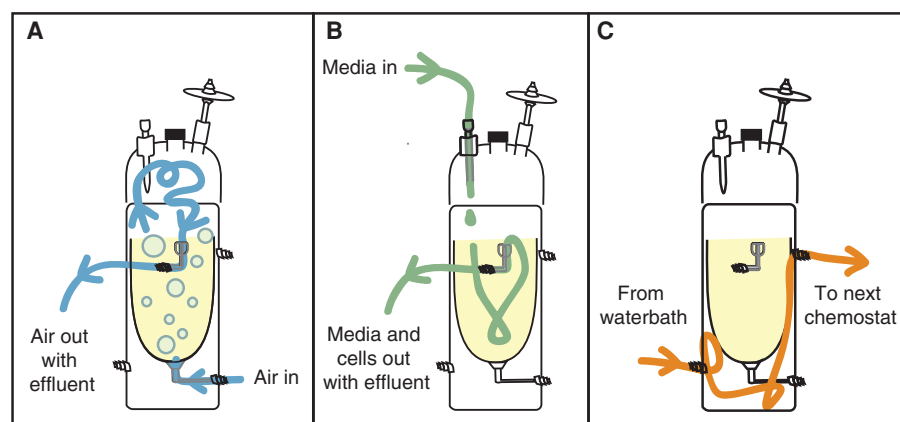


FIGURE 1. Diagram illustrating a basic chemostat. This example of a glass-blown chemostat provides (A) mixing by vigorous aeration, (B) a constant influx of media and overflow of culture effluent, and (C) temperature control via a water jacket.

aeration, and mixing must be optimized to distribute cells and media components uniformly in both space and time. Chemostat platforms can vary in scale over several orders of magnitude with respect to cell population and working volume (a table of vendors and plans can be found in Dunham [2010]). Commercial fermenters that have been modified for chemostat use, such as those from New Brunswick or Infors, typically range from 50 mL to 2 L. Custom glass-blown or otherwise home-built devices typically fall toward the lower end of this range and below (e.g., Klein et al. 2013). Microfluidic implementations weigh in at just a few microliters (Cookson et al. 2005; Groisman et al. 2005; Dénervaud et al. 2013). There are several important considerations to take into account when determining which system scale is most appropriate. Media usage in large fermenters may be prohibitively expensive for long-running experiments and many devices would consume a large laboratory space. However, such a large-scale system might be required to maintain adequate representation of highly complex libraries. At the other extreme, although microfluidic devices help address problems with media consumption and parallelization, population sizes may be insufficient for most evolutionary applications. Run times in microfluidic devices are also most compatible with shorter term physiology and perturbation experiments, although this has been improving (Jakiela et al. 2013).

We have sought a compromise solution to these considerations by using a mid-range 20 mL working volume that can be multiplexed to 32 units in a single array (Miller et al. 2013). For a yeast culture growing at a typical working density, this represents a population size on the order of 10^8 – 10^9 cells. We most typically grow the cultures at a dilution rate of 0.17 vol/h, a rate chosen to sit comfortably below the critical dilution rate at which most laboratory strains switch from mixed respiro-fermentative growth to primarily fermentation when grown in glucose limitation (van Dijken et al. 2000) and comfortably above the point where synchronized metabolic cycling has most commonly been observed (Tu 2005). This dilution rate is also convenient for collecting 1 mL of culture in 15–20 min for practical passive sampling from the overflow port. One 32-chemostat setup costs approximately \$10,000 and fits on a benchtop or in a vertical rack configuration. Although the system is still complex with many parts, it can be operated by appropriately trained and supervised undergraduate researchers.

Our setup does have several limitations in its current implementation. First, all 32 chemostats must be operated off the same peristaltic pump. Running a subset of vessels at different dilution rates can be achieved by changing their working volume, or by using additional pumps that run independently. The off-the-shelf construction also lacks many of the sensors that commercial fermenters are equipped with. New advances in miniaturized sensor technology will hopefully address this limitation. Finally, we have not solved the long-appreciated, platform-independent problems of wall growth and media line colonization. Media lines could conceivably be outfitted with an additional air gap to try to limit this problem, although colonization could still exist at the site of entry. We are currently exploring strain-engineering approaches to abrogate this problem.

Despite these technical issues, the chemostat design presented in Protocol: **Assembly of a Mini-Chemostat Array** (Miller et al. 2015) has been successful in hundreds of experiments lasting an aggregated sum of tens of thousands of generations. We previously showed its equivalence to commercial chemostat platforms with respect to physiology and experimental evolution outcomes (Miller et al. 2013). We hope that our basic chemostat model and detailed protocols for applying the ministat array to problems in yeast physiology (see Protocol: **Chemostat Culture for Yeast Physiology** [Kerr and Dunham 2015]) and experimental evolution (see Protocol: **Chemostat Culture for Yeast Experimental Evolution** [Payen and Dunham 2015]) will encourage additional laboratories to adopt this useful culturing technology for their own applications. These protocols have been optimized to allow utilization of undergraduate researchers working as part of a research team.

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