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Automatic control of growth and density in rotifer cultures

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ABSTRACT

A system for automatic control of the growth and density of rotifer cultures is presented and tested. The system computes feeding rates based on a setpoint for rotifer density, and provides a fast growth period followed by rapid stabilization of the rotifer density. At the same time, overfeeding is prevented, thereby reducing the risk of culture crashes. Feeding rates are automatically computed based on measurements of the culture's density and egg rate, and internal setpoints for growth rate and egg rate. The controller is tested and tuned against a mathematical model of a rotifer culture before being applied to four experimental cultures.

The experimental results show densities in all tanks increasing from 60 to 90 ml^{-1} to the setpoint densities of 500 and 1000 ml^{-1} in 5–7 days, after insignificant growth on the first day. Gross growth rates slowed down considerably towards the end of the experiment, as the controller reduced feed rations in order to stabilize densities. The cultures were stabilizing at higher densities compared to their setpoints, while there was no such bias in the observed egg rates. This indicates that a part of the controller algorithm needs further tuning. The approach to the setpoint densities was fast, and within the experimental period there were no indications of oscillations beyond those caused by daily dilution.

1. Introduction

In the initial period after hatching, larvae of most marine fish species require live food, and this is part of the reason why the larval stage is a bottleneck in the development of marine aquaculture (Kolkovski et al., 2004). Rotifers of the species complex *Brachionus plicatilis* are used as live food for most marine species. Production methods for rotifers include batch cultures, semi-continuous cultures with daily harvesting and dilution, cultures with continuous harvesting and closed recirculation systems (Dhert et al., 2001). Marine hatcheries typically employ a fairly low level of automation, and some recent papers have addressed the use of automation in the rotifer period (Papandroulakis et al., 2002; Kolkovski et al., 2004; Alver et al., 2007, 2008). However, there is still great potential for exploiting automation techniques, which have contributed to improved predictability and cost reductions in other process industries (Haley and Mulvaney, 1995; Jämsä-Jounela, 2001).

In order to reduce the amount of manual work, and improve the stability of rotifer production cultures, we aim to implement a control system using the feeding rate as an input to steer the rotifer density of the cultivation tanks towards a reference density. We believe that the stability of rotifer cultures is improved by carefully controlling feed rations based on up-to-date information about the culture's state, thereby adapting feeding rate to the feed requirement as closely as possible. According to Olsen (2004, p. 97), a disparity between food ration and food consumption may be the most common problem in rotifer cultivation, particularly during the critical early growth phase, because of occasional overfeeding or starvation. The key to controlling culture growth and density optimally is to understand the dynamics of the culture, and this can be facilitated by studying a mathematical model of the system.

The growth rate of a rotifer culture is a function of input variables such as the concentration and quality of feed, the water temperature and salinity, and internal states such as the egg rate and age structure of the rotifer population. The production and hatching of an egg can take 1–2 days (Korstad et al., 1989; Dhert, 1996), thus introducing a delay between changes in the input values and the response in the growth rate. The general effect of a transport delay in a closed-loop dynamical system is to introduce or increase oscillations in the system output, and one must therefore take care to avoid instability when applying a controller.

The transient dynamics of rotifer cultures cannot be well described by classical chemostat models developed for unicellular organisms, as shown by McNair et al. (1998). This problem is solved

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Table 1
List of the symbols used in the controller equations.

Symbol	Unit	Description
D	day^{-1}	Dilution rate
E		Egg rate
$\hat{E}$		Measured egg rate
E_r		Reference egg rate
$g(u)$		Egg rate as function of growth rate
$g^{-1}(E)$	day^{-1}	Growth rate as function of egg rate
$h(E)$	μg day^{-1}	Individual feed ration as function of egg rate
κ_u		Correction factor to U_b
K_{I1}	day^{-1}	Integrator coefficient for growth rate
K_{I2}		Integrator coefficient for egg rate
K_{P1}	day^{-1}	Proportional coefficient for growth rate
K_{P2}		Proportional coefficient for egg rate
$\hat{\mu}$	day^{-1}	Estimated growth rate
μ_D	day^{-1}	Growth rate needed to compensate for dilution
μ_r	day^{-1}	Desired culture growth rate
q_u	ml min^{-1}	The pumping rate of feed
R	ml^{-1}	Rotifer density
$\hat{R}$	ml^{-1}	Estimated rotifer density
$\hat{R}_p$	ml^{-1}	Projected rotifer density
R_r	ml^{-1}	Reference density
ρ_{res}	g ml^{-1}	Feed density in reservoir
Δt_d	days	Dilution interval
u	g l^{-1} day^{-1}	Daily feed ration
U	s	The feed pumping time per cycle
U_b	μg day^{-1}	Estimated feed ration corresponding to E_r
u_{cycle}	g	The amount of feed to add per pumping cycle
U_u	μg day^{-1}	Individual feed ration
V_w	l	Tank volume

by utilizing an individual-based model such as the one presented by Alver et al. (2006), which does not depend on assumptions of steady-state conditions.

In this study, the model of Alver et al. (2006) will be used as a test system for the initial tuning of the controller. The model is used to simulate a rotifer culture, using feeding rates calculated by the controller. The controller uses rotifer density and egg rate calculated by the model as basis for calculating the input. In this way, the controller is tested with the model serving as a proxy for the real system.

2. Materials and methods

2.1. Concept

All symbols introduced in the following are listed in Table 1. We consider a system consisting of a semi-continuous rotifer culture and its inputs and outputs (Fig. 1). The main input value affecting population growth is the feeding rate. Feeding is handled using a controller unit which uses measurements on the rotifer culture to determine the required feeding rate. Algae in the form of algae paste is used as feed, although the system can also be applied to other feed types. The controller's purpose is to ensure a rapid increase of the rotifer density, R [ml^{-1}], up to a reference level R_r, and thereafter hold the density close to this level. This should be achieved while minimizing the amount of feed used, and minimizing the risk of a culture "crash" (meaning sudden massive deaths leading to a population decrease) by continuously adapting to the current culture density, by optimizing feed rations to avoid overfeeding or starvation, and by preventing the density initially shooting far above the desired level. These measures can be expected to reduce the risk, although crashes can also have other causes (Dhert et al., 2001; Papakostas et al., 2006).

The controller achieves this by relying on measurements of the state of the rotifer culture. The most important measurement is that of the rotifer density, being the primary output value that we wish to control. The density can be measured manually by counting individuals in preserved samples using a stereo microscope. However, manual counting is time consuming, and vulnerable to subjective bias depending on who is doing the counting. For instance, sources of error such as the choice of sampling location, the accuracy of the volume measured out by the pipette, and the determination of which particles to count will depend on the person making the measurement. An automatic rotifer counter has been developed, which allows frequent measurements, shows high accuracy, and resolves the issues of bias and manual work (Alver et al., 2007).

The average number of eggs carried by each female (the egg rate) is another important variable in a rotifer culture (Olsen, 2004, p. 79). The egg rate is a strong predictor of the growth rate of the culture in the nearest 24 h, and for this reason a measurement of the egg rate is of significant value when computing the feed input for the culture. When manually measuring rotifer density, the eggs carried by each individual are visible and can be counted in order to determine the egg rate.

2.2. Estimation of feed and growth dynamics

Before outlining the controller design, we use the model of Alver et al. (2006) to estimate a number of key functions defining the feed and growth dynamics of a rotifer culture. The function $h(E)$ should give the required individual feed ration as a function of the desired egg rate, and $g(\mu)$ the steady-state egg rate as a function of the culture growth rate μ_r.

The functions are estimated by running the model at steady-state with constant feed addition and a series of dilution rates. At steady-state the population density is constant, and growth rate depends on the dilution rate only. We get the corresponding values for the specific feed ration [g wet weight ind^{-1}] by dividing the feed addition rate by the number of individuals for each dilution rate, and the egg ratio can be extracted directly from the model. The values are shown in Fig. 2. Using MathWorks Matlab 7.2, the polynomial functions $h(x)$ and $g(x)$ were fitted to the data by least squares estimation:

$$h(E) = 6.38 \times 10^{-6}E^3 - 7.72 \times 10^{-7}E^2 + 1.69 \times 10^{-6}E + 5.80 \times 10^{-7} \quad (1)$$

$$g(\mu) = 1.50\mu^2 + 0.89\mu + 0.065 \quad (2)$$

We also need an estimate of the growth rate as a function of egg rate. This is the inverse function of $g(E)$, and can be approximated

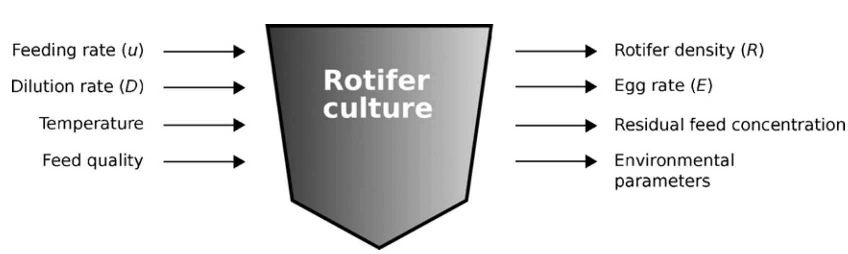

Fig. 1. Overview of the system inputs and outputs.