

A Kalman filter for estimating enzyme levels during biphasic growth

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Biological nitrogen removal in wastewater treatment plants is achieved through a set of alternating bacterially mediated redox reactions. Under aerobic conditions, ammonia is oxidized to nitrate by aerobic autotrophs. In the absence of oxygen, denitrifiers will reduce nitrate to nitrogen gas. The culture must be cycled through both aerobic and anoxic conditions in order to accomplish complete denitrification. However, the denitrifiers are also facultative anaerobes, and will grow during the aerobic phase. During this time they will not fully express the enzymes necessary for nitrate respiration, and the specific enzyme level will decrease due to enzyme decay and dilution due to growth. There will be insufficient nitrate generation if the aeration period is too short, but if it is too long the denitrifiers' nitrate respiration enzyme levels will become very low. If that happens, the denitrifiers will experience a diauxic lag when the reactor returns to anoxic conditions. This implies that there is an optimal duration for the aerobic phase.

Current wastewater plant operation is often suboptimal, using a fixed aeration period regardless of load. A dynamic optimization scheme would need to incorporate nitrate respiration enzyme levels in order to determine the impact of aeration on the upcoming anoxic phase. Enzyme measurements are problematic in that they cannot be performed online. In order to address this problem, we have developed a model for biphasic (also called diauxic) growth of denitrifiers and combined this model with a Kalman filter to generate online estimates for nitrate reductase activity. Using this information, a control scheme could be constructed which would be cognizant of the diauxic lag resulting from excessive aeration.

Diauxic growth occurs when a preferred growth substrate is exhausted and a period of little or no growth occurs. During this period necessary enzymes are synthesized that allow growth on the less preferred substrate. Diauxic growth was first characterized for the case of changing electron donors (Monod, 1942), and was later observed to occur when switching terminal electron acceptors (Kodama et al., 1969). Several investigators (Gouw et al., 2001; Lisbon et al., 2002; Liu et al., 2000, 1998; Waki et al., 1980) have studied the particular diauxie occurring when bacteria switch from oxygen to nitrate, which is known to occur with biomass from an activated sludge process used

for denitrification (Liu et al., 1998). Traditional models of single-sludge processes, such as the IWA Activated Sludge Models (ASM) 1, 2, 2d and 3 (Henze et al., 2000) do not predict this effect.

We have developed a model (Figure 1) for diauxic growth of denitrifying bacteria in which nitrate reductase synthesis plays the key role in determining overall denitrification kinetics. Our approach models diauxic lag as resulting primarily from nitrate transport limitation. This was achieved by developing a model structure that uses intracellular nitrate as the inducer for an operon coding for nitrate reductase and nitrate transport enzyme (Figure 2). This approach was successful in fitting data on biomass from the literature, as well as data on biomass growth and enzyme data collected as part of this study (Figure 3). The nitrate reductase synthesis dependence and coupled nitrate transport limitation explains the dependence of lag length on aeration time, the cessation of anoxic growth in the presence of oxygen, as well as the observed nitrate reductase enzyme activity profile during diauxie. Thus it may be concluded that a model based on enzyme biosynthesis regulation can be successfully applied to portraying diauxic growth due to switching of terminal electron acceptors.

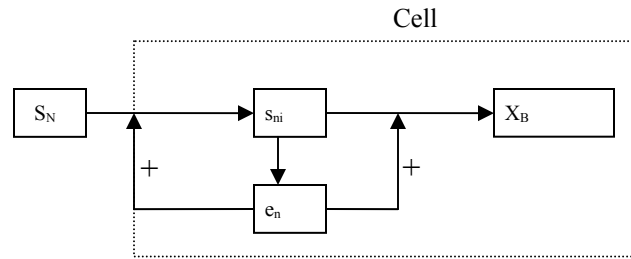


Figure 1. Model overview. Uptake of nitrate, S_N , results in an increased level of internal nitrate, s_{ni} . This promotes synthesis of nitrate reductase, e_n , which in turn promotes synthesis of new biomass, X_B , and increases the rate of nitrate uptake.

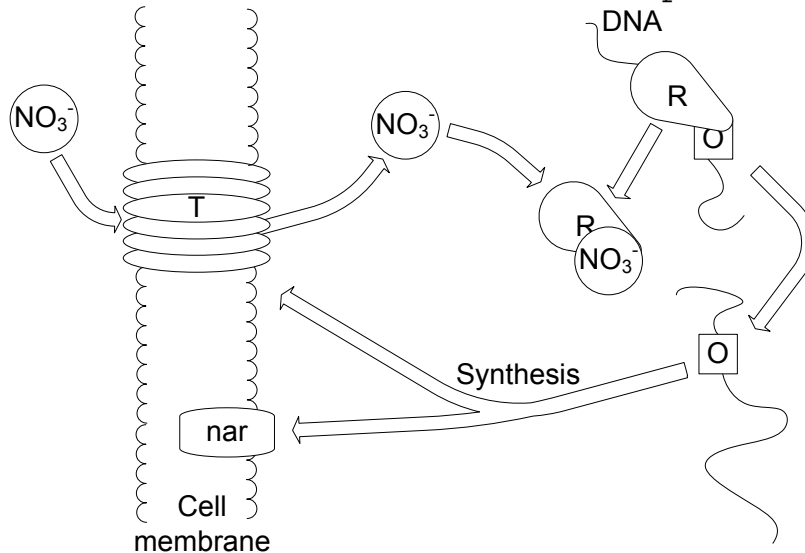


Figure 2. The biochemical process being modeled is that nitrate (NO_3^-) is actively transported into the cell by transport protein T. Internal nitrate binds to repressor R, freeing operator O. Synthesis of the nitrate reductase, nar, and transport protein proceeds at a

rate proportional to the amount of free operator. We assume the existence of a nitrate respiration operon, and that the transport protein and nitrate reductase are therefore synthesized together.

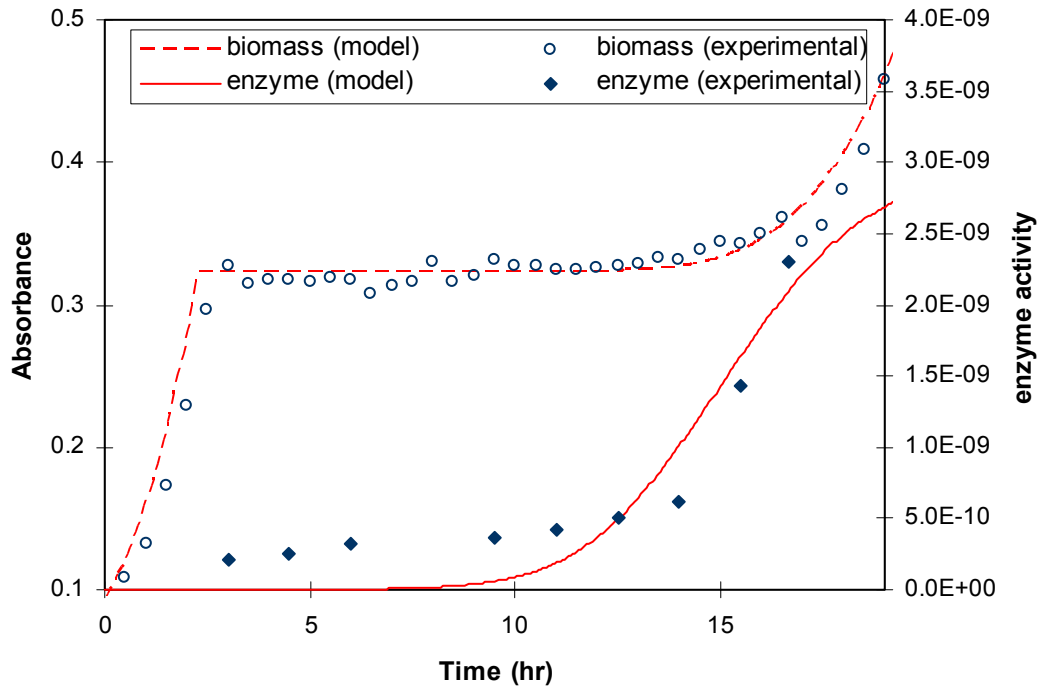


Figure 3. Data points show experimental results; dashed and solid lines show model fits.

We applied a Kalman filter to this biochemical model, which allowed us to generate online estimates (Figure 4) of nitrate reductase activity levels based on frequent biomass measurements and less frequent enzyme activity measurements. In a plant environment, these estimates could be made based on all available on-line measurements (biomass density, nutrient levels) as well as periodic off-line enzyme activity measurements. These resulting enzyme estimates would then be used in a dynamic optimization scheme for control of aeration period length in the face of changing plant loading. The aeration length must be such that nitrate respiration enzymes do not fall so low as to cause a significant lag in the subsequent anoxic phase.

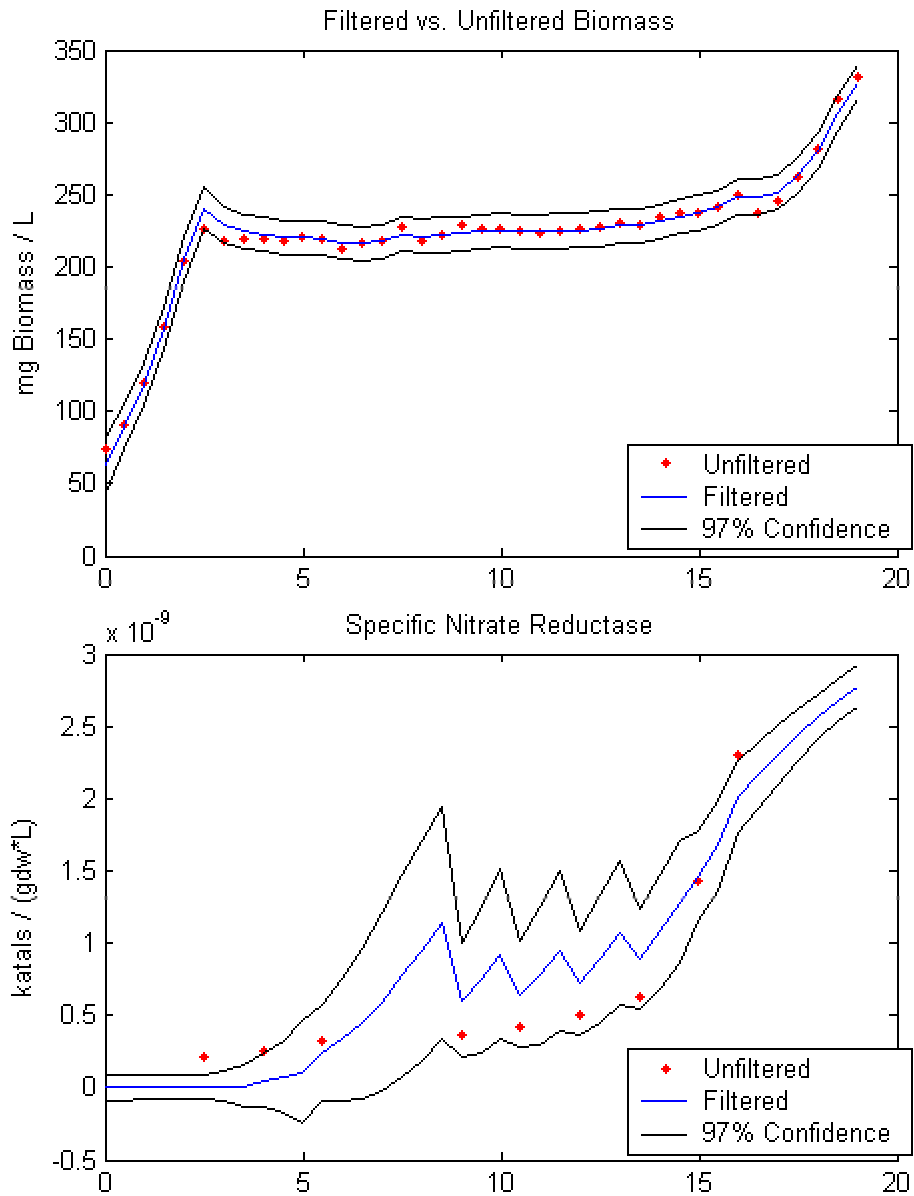


Figure 4. Kalman filter performance. Solid black lines represent filter estimate confidence, as extracted from the diagonal of the error covariance matrix.

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