

# Mixotrophic Growth of *Chlorella protothecoides* for Wastewater Treatment

## Effects of Retention Time on Membrane Harvesting, Treatment, and Productivity



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### Introduction

This study investigates integration of mixotrophic algaculture at wastewater treatment facilities, specifically the effects of retention time (RT) on biomass productivity, harvesting by membrane filtration, and treatment.

Membrane filtration was chosen as the harvesting method to:

- Ensure exclusion of problematic algal blooms from other treatment operations in the proposed system
- Understand the impacts of bioreactor operational controls on membrane fouling

Semi-continuous cultures, with once daily harvesting and feed addition, were used. Solids and hydraulic retention times (SRT and HRT, respectively) were equal in each reactor (Figure 1).

*Chlorella protothecoides* was chosen due to its natural occurrence in engineered wastewater systems<sup>1</sup> and its lipid accumulating characteristics<sup>2</sup>.

### Harvesting

Fifty milliliters (50 mL) from each reactor were harvested using 0.22  $\mu\text{m}$  cellulose acetate membranes in a stirred, dead-end filtration cell (Amicon 8050) and flux was monitored (Figure 2). Flux recovery was evaluated as percent of initial clean-water flux after harvesting and rinsing with distilled water (Figure 3). Energy inputs were also evaluated. Results showed:

- Increasing RT correlated to increased energy input for harvesting
- Cleaning was more effective at lower RTs, resulting in higher flux recovery

### Productivity and Treatment

Biomass productivity and removal of COD were analyzed using measurements of soluble (0.22  $\mu\text{m}$  filtered) and total (soluble + biomass) COD (Figure 4), and were compared to DOC.

Results showed:

- Complete removal of COD (<6 mg/L effluent) was achieved at all RTs
- Biomass productivity was largely a function of organic carbon loading
- The greatest carbon fixation, as a percent of total biomass, was achieved with a 5 day RT (Figure 5). This reactor also resulted in the lowest DOC (data not shown).

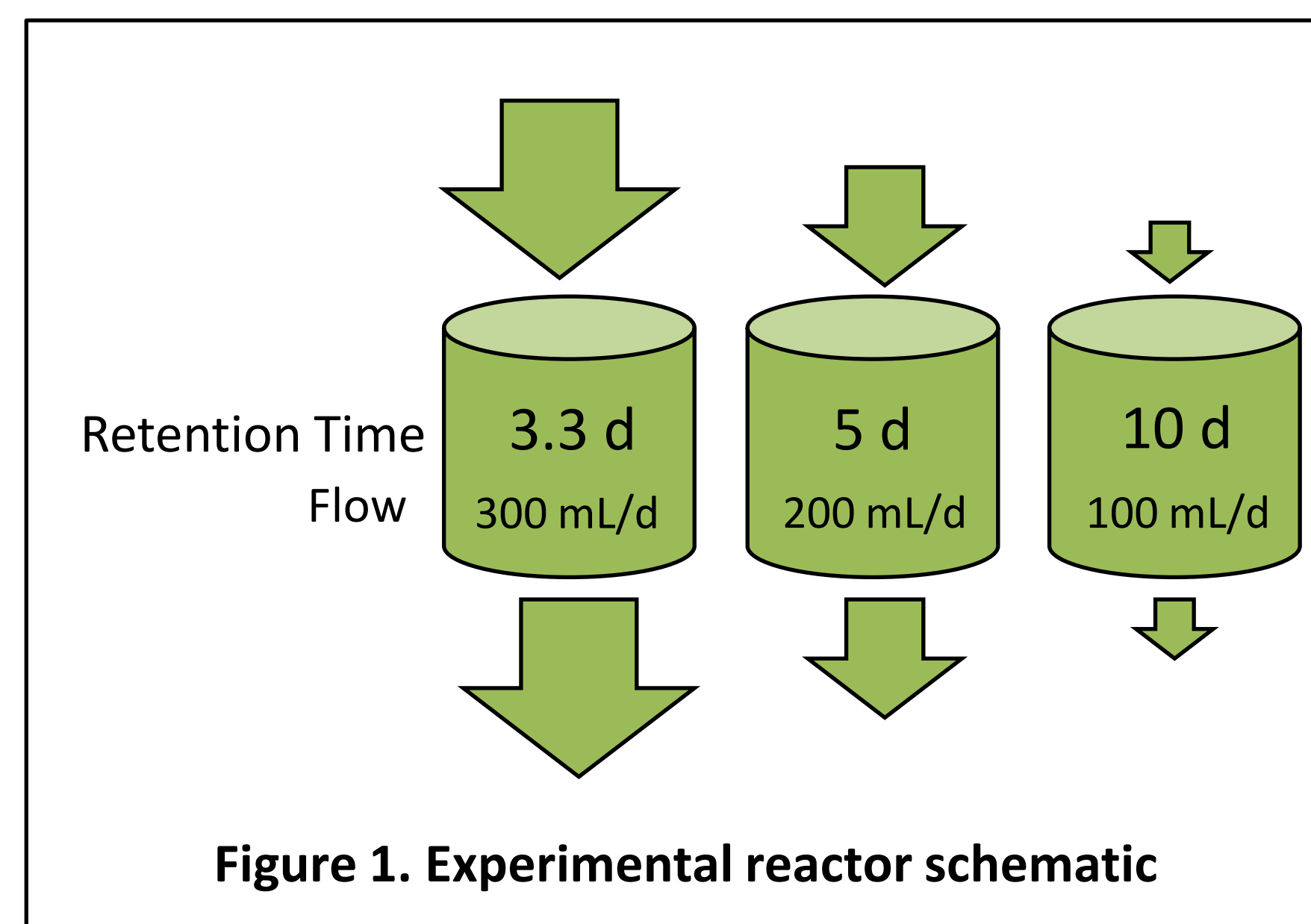


Figure 1. Experimental reactor schematic

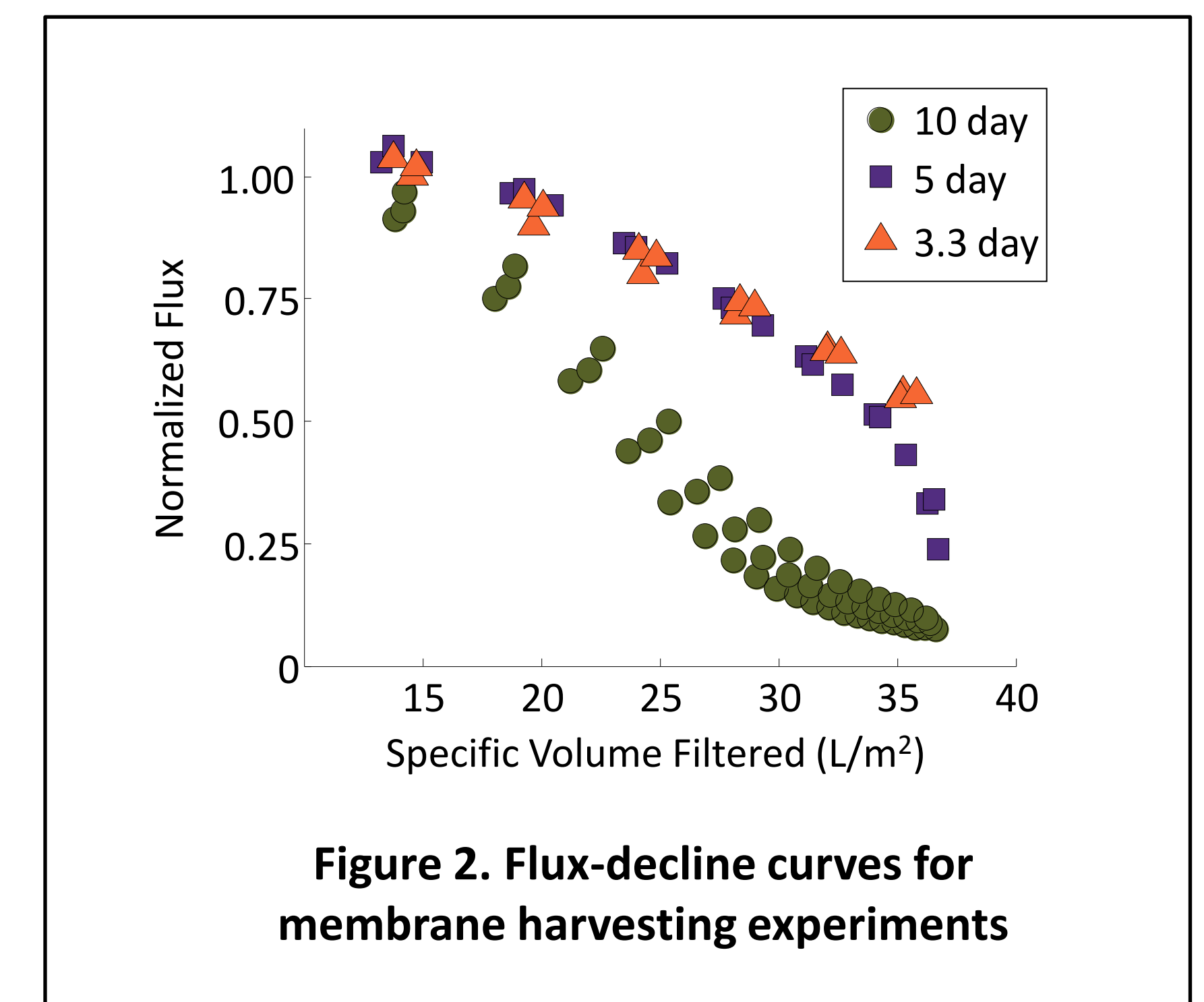


Figure 2. Flux-decline curves for membrane harvesting experiments

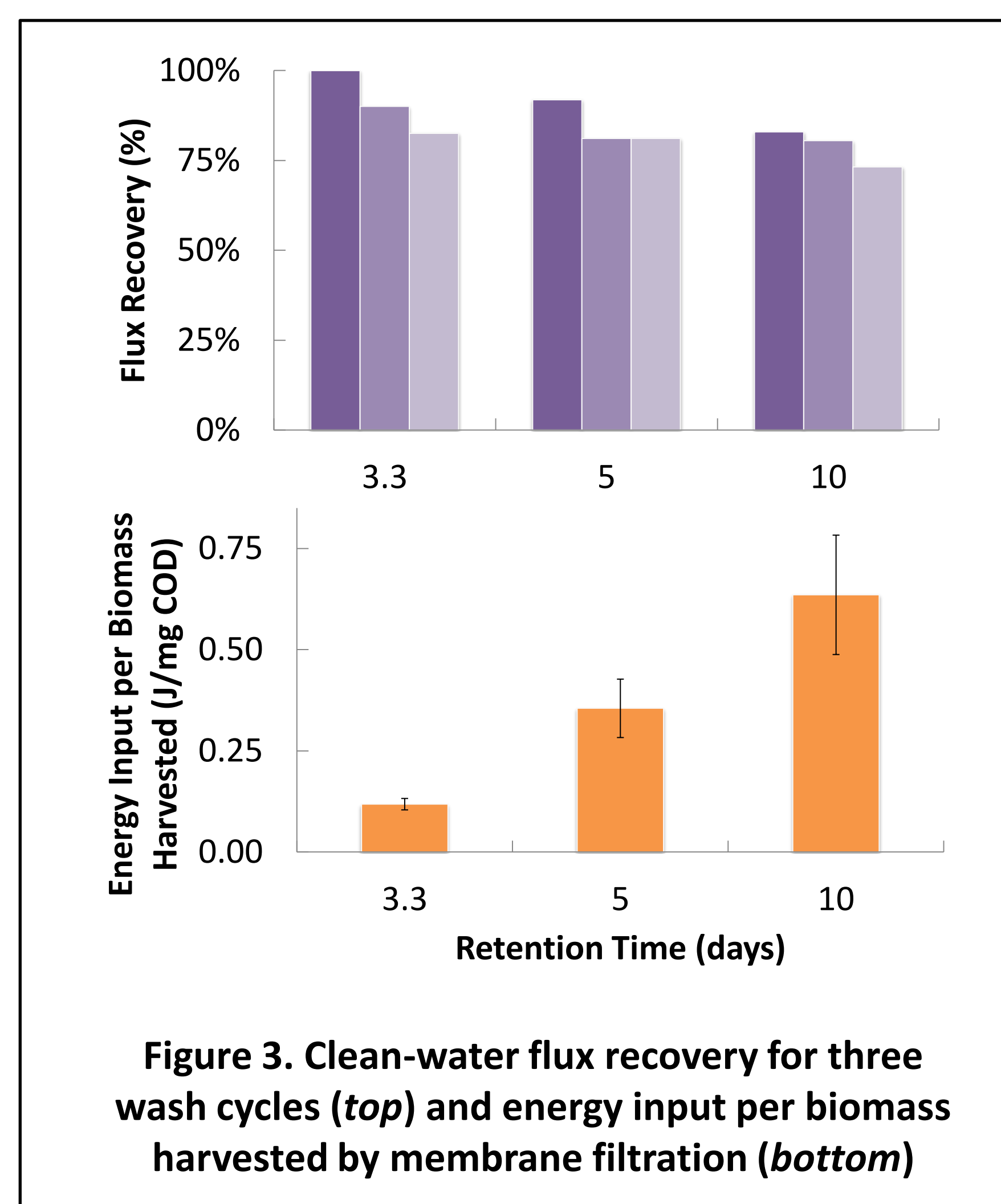


Figure 3. Clean-water flux recovery for three wash cycles (top) and energy input per biomass harvested by membrane filtration (bottom)

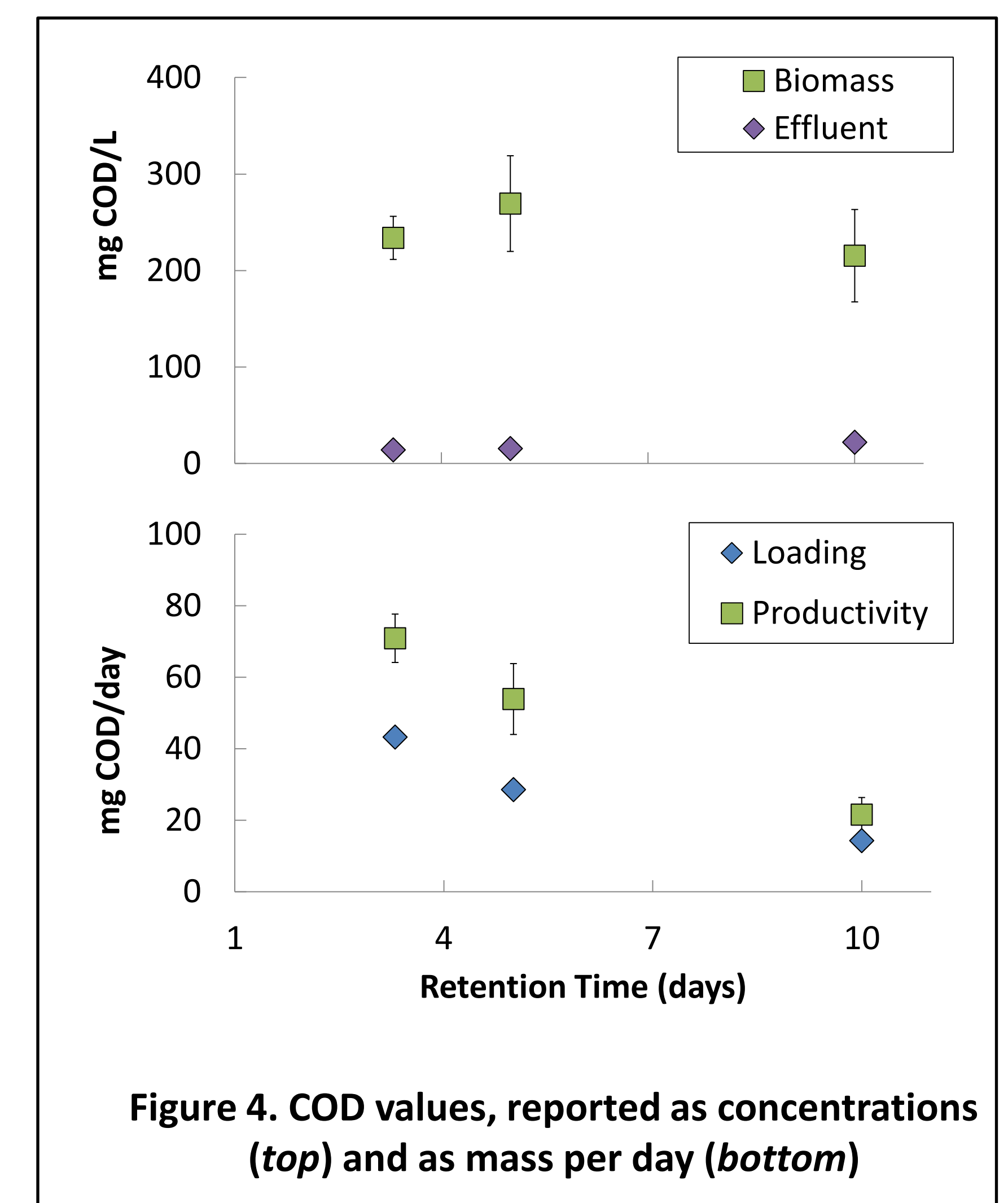


Figure 4. COD values, reported as concentrations (top) and as mass per day (bottom)

### Conclusions

- Treatment of organic carbon by *C. protothecoides* through mixotrophic growth is possible in semi-continuous bioreactors where macronutrient (N, P, etc.) concentrations are not limiting.
- In the presence of a free source of organic carbon (i.e. wastewater), lowering the retention time in algal bioreactors is beneficial for membrane harvesting efficiency and biomass productivity.
- Maximum carbon fixation may be achieved by balancing culture density (a function of solids retention time) with organic carbon loading (a function of waste flow and concentration).

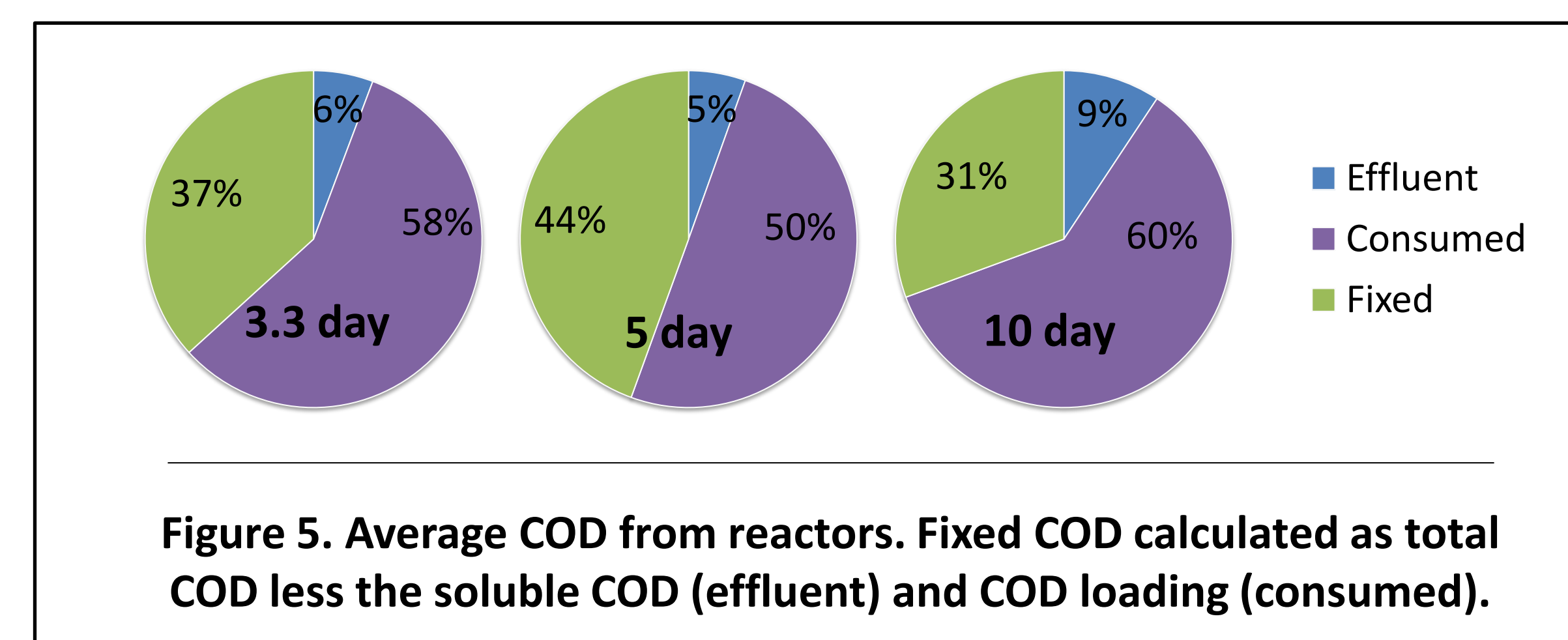


Figure 5. Average COD from reactors. Fixed COD calculated as total COD less the soluble COD (effluent) and COD loading (consumed).

<sup>1</sup>Pittman, J.K., et al., 2011. The potential of sustainable algal biofuel production using wastewater resources, *Bioresource Technology*.  
<sup>2</sup>Chen, Y.H. and Walker, T., 2011. Biomass and lipid production of heterotrophic microalgae *Chlorella protothecoides* by using biodiesel-derived crude glycerol, *Biotechnology Letters*.

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