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Project Overview

CH₄ and CO₂ emissions from dairy operations constitute ~2.5% of annual U.S. greenhouse gas (GHG) emissions, making dairies one of the largest industrial sources of GHGs in the U.S. Anaerobic digestion (AD) of dairy manure can reduce dairy CH₄ emissions while producing electricity. However, dairy ADs are severely constrained economically, principally due to low electricity rates. Our project is designed to develop novel, integrated strategies to achieve a net reduction in dairy GHGs while producing value-added commodities. Commodities include bio-gas (CH₄), PHA-based bioplastics, carbohydrate production via algal polyculture for enhancing PHA production, and nutrient management also via optimizing PHA reactor conditions and algal polyculture. Additionally, we are developing an integrated system model that will provide a decision making tool for researchers, AD system developers/operators, and dairy owners. Lastly, we are expanding education in integrated agriculture systems for economically viable GHG management.

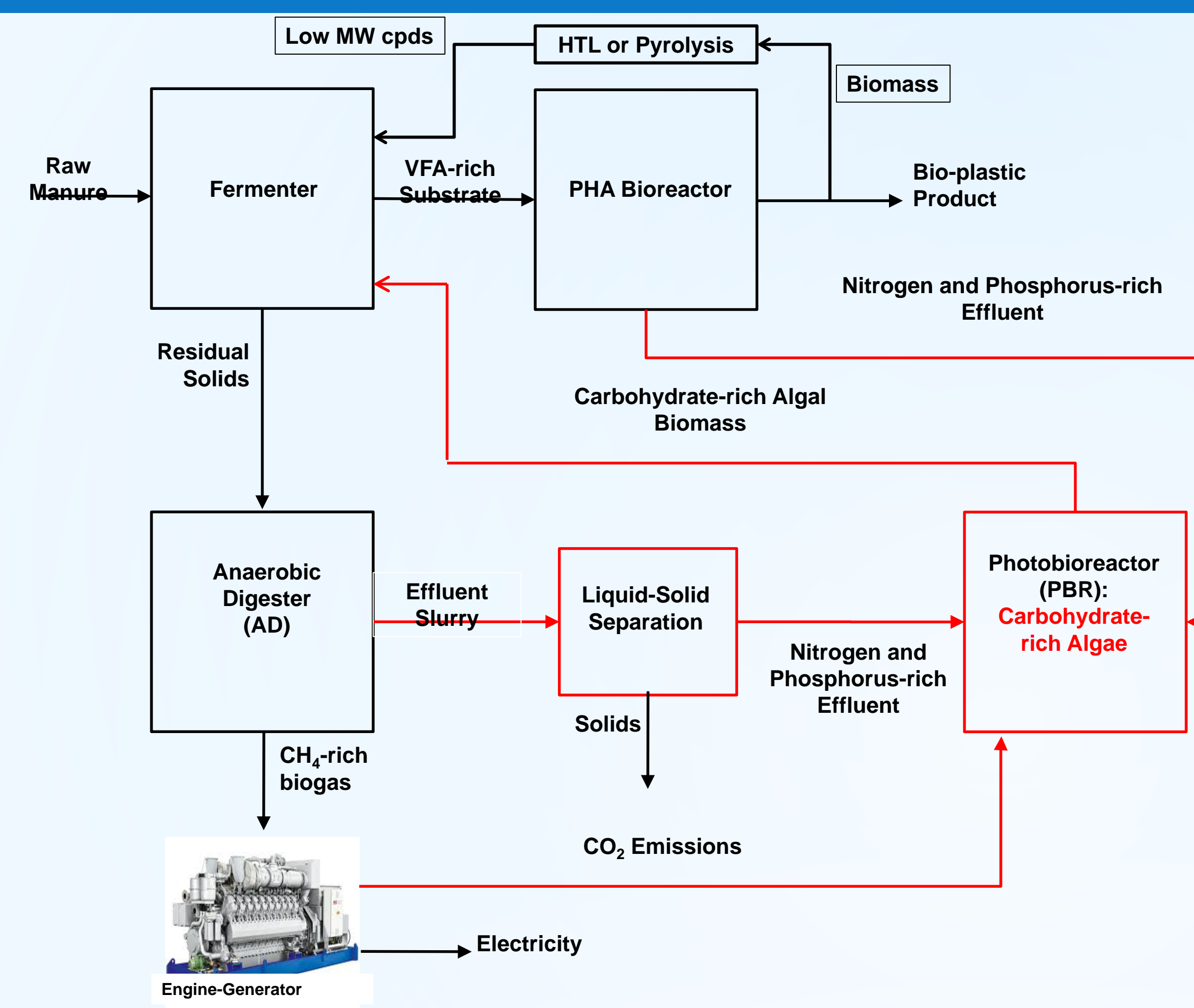


Figure 1-Integrated Dairy Manure Commodities Process: Focus on Algae Process

Project Objectives: Focus on Algae, System Integration, Outreach

- GHG MITIGATION and PRODUCTION OF VALUE ADDED COMMODITIES:** To enhance dairy carbon (C) sequestration, this project is advancing a novel integrated manure-to-commodities system that (iii) sequesters AD effluents (CO₂, nitrogen, phosphorus) by producing algae that can be harvested and internally recycled to enhance PHA production and enhance overall C-sequestration (Fig. 1). Optimization of the algal process is being pursued as a function of algal species diversity for enhancing net algal carbohydrate production and resistance to microbial grazers.
- SYSTEM INTEGRATION:** GHG reduction and C sequestration will be quantified and used to parameterize a system model and web-accessible management decision tool (DAIRIEES) that will be developed by the Idaho National Laboratory.
- EDUCATION:** Produce the next generation of scientists/engineers, and educate young scientists in the importance of integrated agricultural systems. Dissemination of research products and decision tools along with student training will be facilitated by delivering a Manure to AD to Algae education and experimentation module for K-12 educators that we have developed. Thus far we have involved approximately 1800 K-12 educators in this activity via the Idaho iSTEM education institute (2013-2014). Currently, all of the 7th grade science educators in the Caldwell, ID school district have adopted this module for a 4 week section on algae cultivation. Student experiment results will be presented at the Caldwell science fair. A large percentage of these students come from underrepresented groups in STEM.

Research Progress

Algae production: From AD/PHA effluent to a carbon and nutrient sink

The Grand Algae Challenge: How to optimize productivity and stability?

Productivity: Increased algal productivity is necessary to enhance economic viability and improve nutrient sequestration of algal systems.

Stability: US DOE cites process stability of open systems and grazer control as one of main research needs for algal cultivation in wastewater systems.

Our Solution: Algal diversity and active grazer management.

Why Diversity? Diversity-productivity-stability relationships are present in a variety of ecosystems. Greater diversity can result in resource use complementarity which, in turn, drives increased productivity. Interference effects can lead to reduced grazing rates and increased system stability (a.k.a. process stability) over time.

Our approach:

- Step 1:** Determine which species grow well in ADE and PHAE (data not shown, strains included in Table 1 represent experimental outcomes).
- Step 2:** Determine diversity effects and which species produce more as a mixture than they do as monocultures (data shown here).
- Step 3:** Assess process stability in continuous culture at bench and pilot scales (experiments on-going).

Step 2b: Test productivity, stability, and resistance to grazing pressure of top performing cultures in larger volume cultures with pH control and CO₂ addition

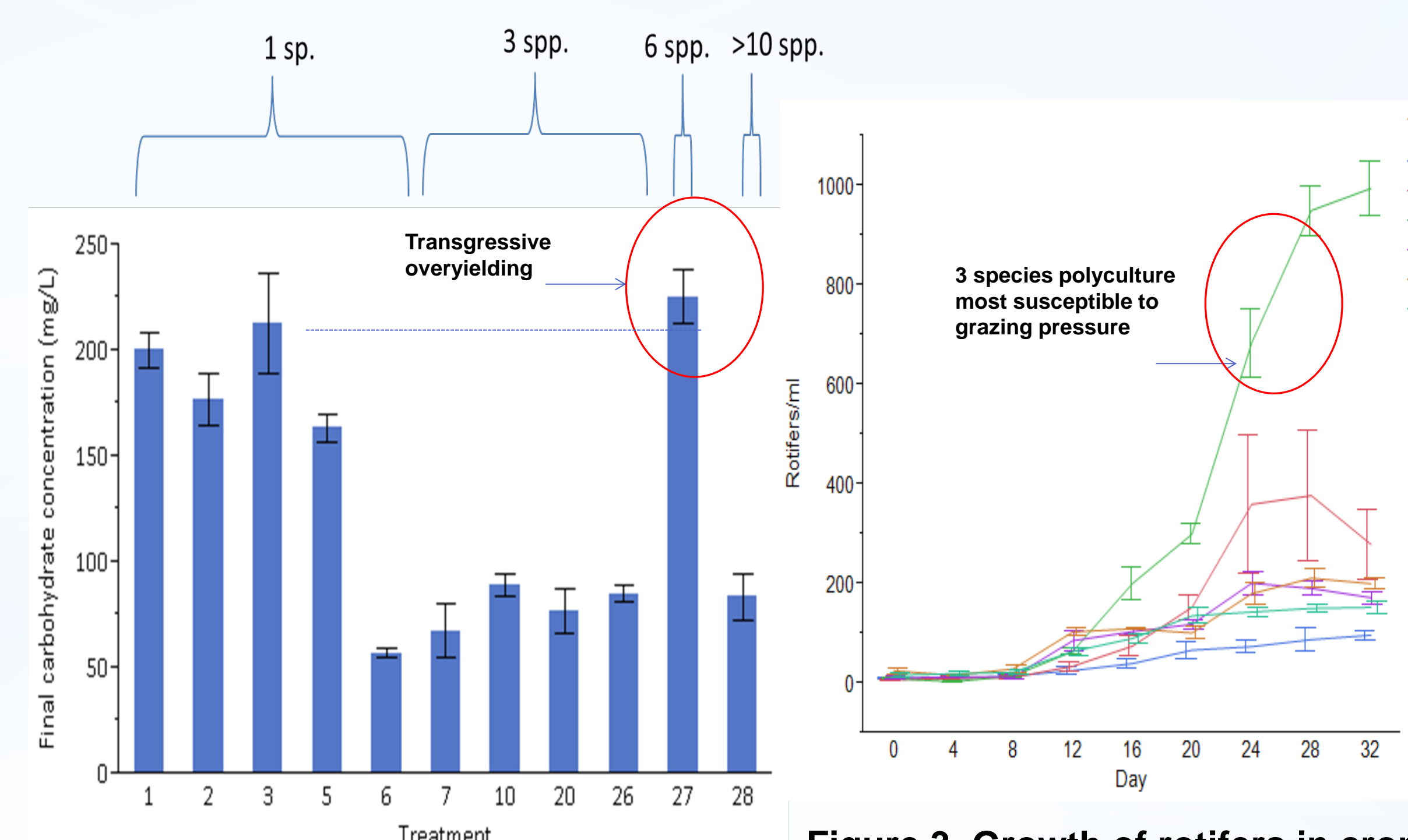


Figure 2. Final carbohydrate levels in top performing single species and mixed algal cultures. Larger volume experiments with pH and CO₂ control reduce diversity effects, but still result in transgressive overyielding for some polycultures.

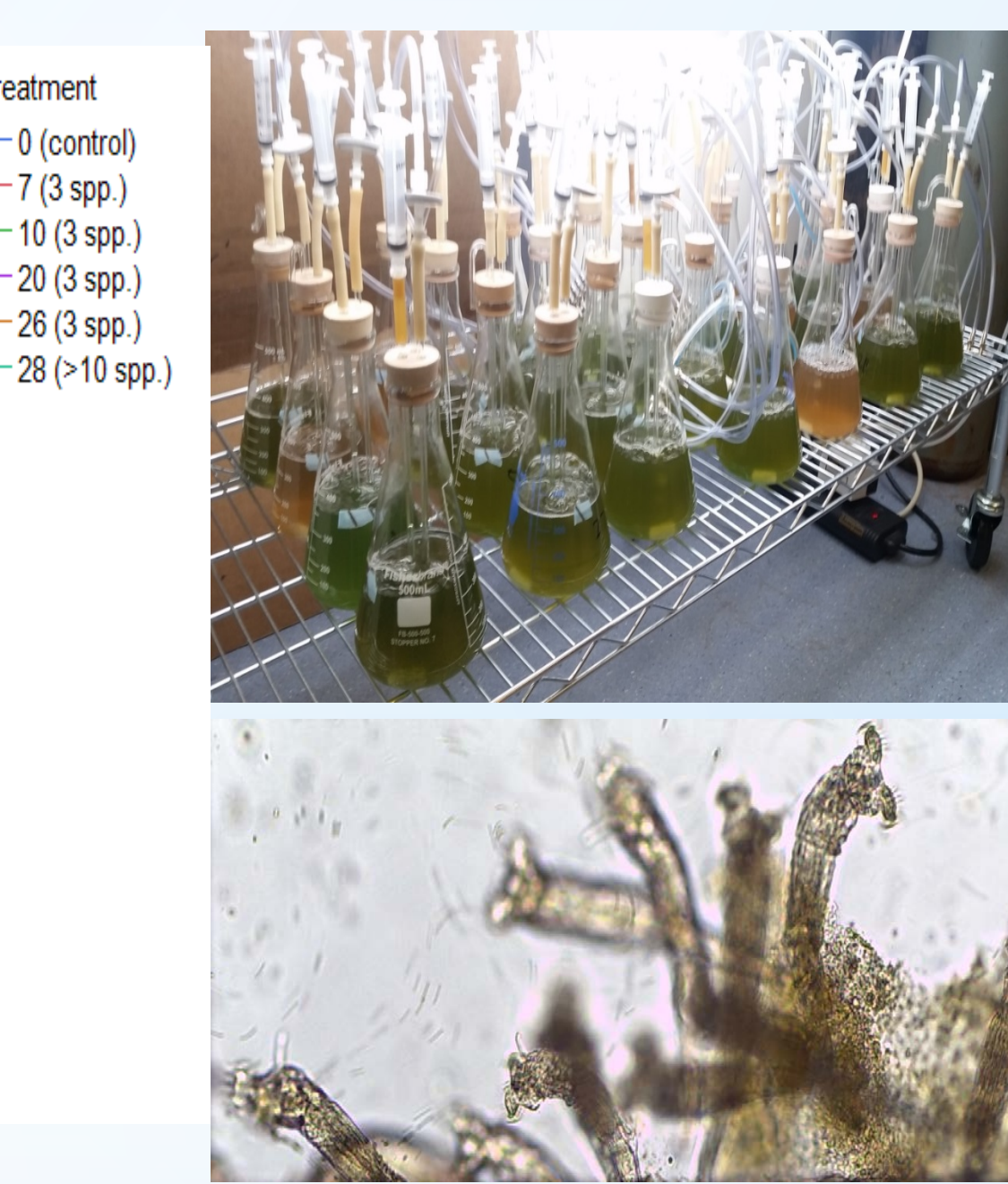


Figure 3. Growth of rotifers in crop protection experiment to identify polycultures with higher resistance to grazing. Increased grazer resistance can lead to increased system stability in open systems vulnerable to contamination by pest species.

Step 3: Assess continuous process stability at pilot scale



Figure 4. A) Experimental setup for Step 2b. Each treatment combination was run in quadruplicate (n = 4). B) Photo micrograph of rotifers feeding on a monoculture.

Figure 5. Overyielding polycultures or those with high levels of grazer resistance are being transitioned to pilot scale raceways (200L) for large-scale continuous cultivation trials. These experiments are planned for Summer 2015.

1) Results from these and the smaller volume experiments are being used to populate the DAIRIEES Model.

Step 2a: High throughput, small volume screening of algal polycultures

Table 1. Key to the species composition of all polycultures tested. Algae and cyanobacteria strains were chosen based on published carbohydrate production rates.

CR	<i>Chlamydomonas reinhardtii</i>
CV	<i>Chlorella vulgaris</i>
CM	<i>Chlorococcum sphaerosum</i>
MA	<i>Microcystis aeruginosa</i>
SO	<i>Scenedesmus obliquus</i>
SL	<i>Synechococcus leopoliensis</i>

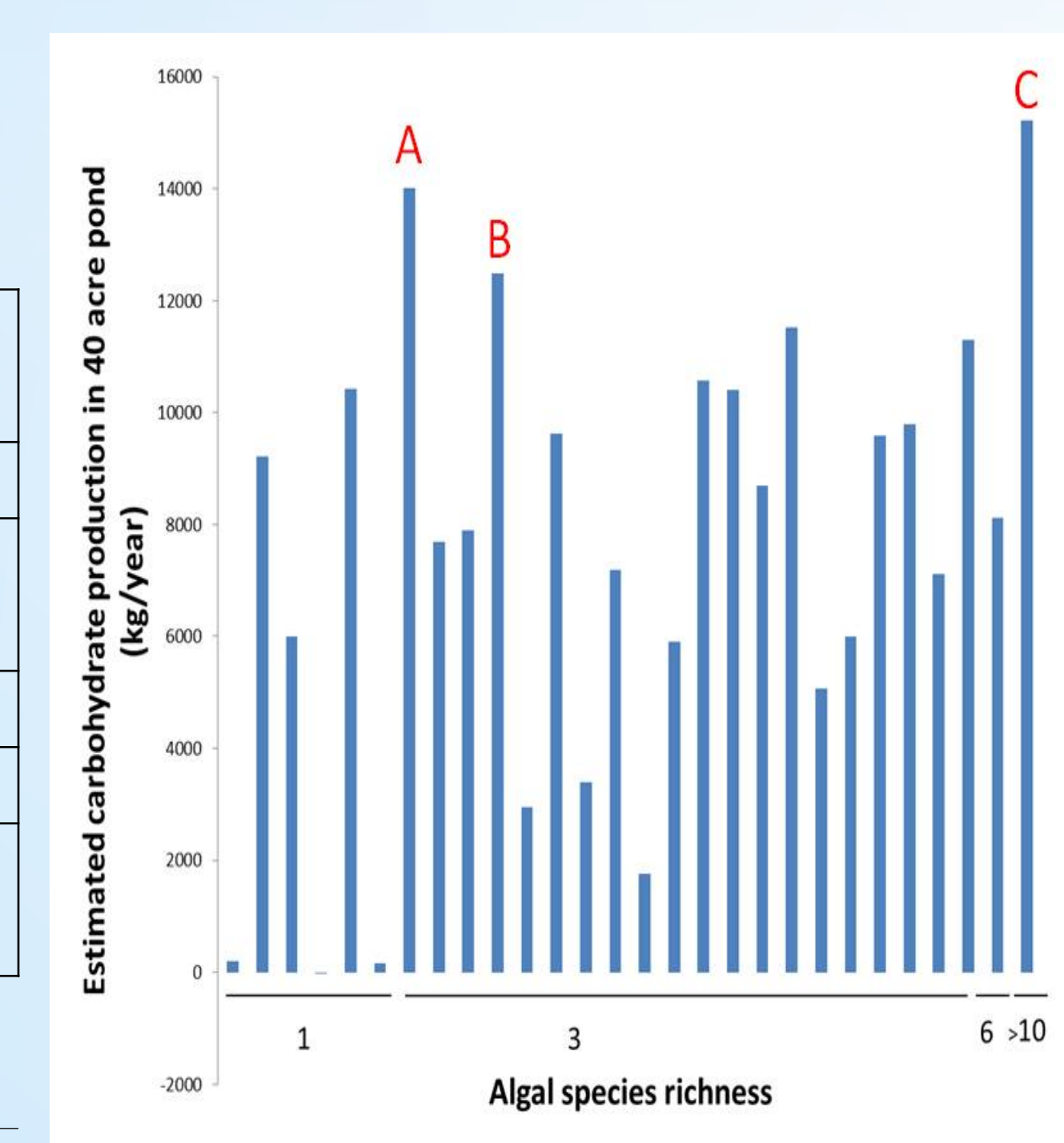


Figure 1. Estimated annual carbohydrate production in a 40 Acre pond (kg/year). Estimates of annual yields are based on logistic growth rates and asymptotic biomass levels. The most productive polycultures in this experiment were the following:

- A:** *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Chlorococcum sphaerosum*
- B:** *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Synechococcus leopoliensis*
- C:** Diverse natural algal polyculture collected from Boise River

Initial conclusions:

- Top performing polycultures increased carbohydrate yield by 46%.
- Specific species/strain composition controls yield.

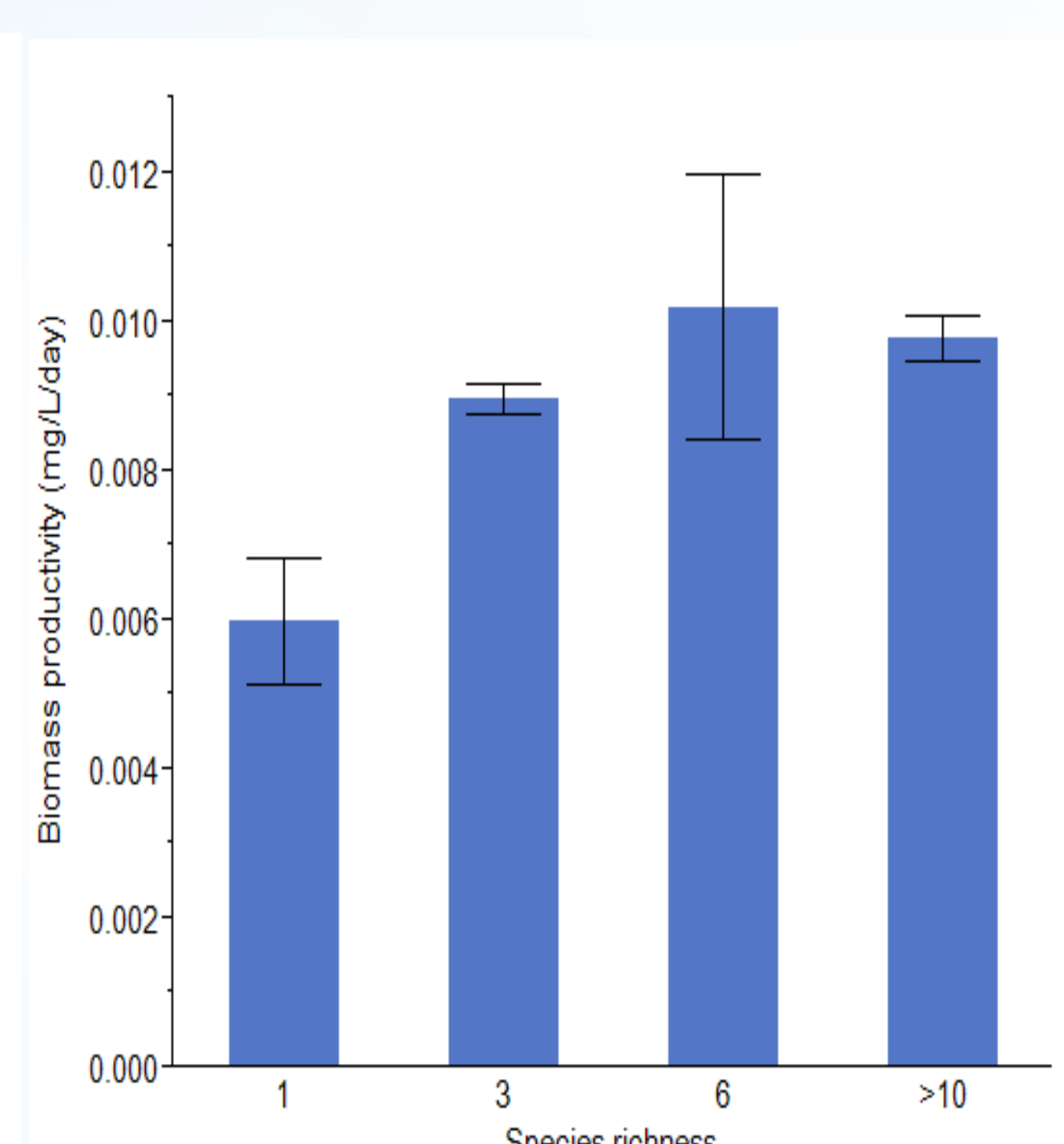


Figure 2. Species richness effects on biomass productivity. Final biomass of each treatment was expressed as a function of species richness (range = 1, 3, 6, >10 (for the Boise River algal community)).

Initial conclusions:

- All polycultures had greater productivity rates than the monocultures
- Biomass productivity increased linearly with increasing species richness, for richness levels between 1 and 6.
- Species richness > 6 did not further improve biomass productivity rates.
- Top performing polycultures produced annual biomass yields 60% higher than the best monocultures.

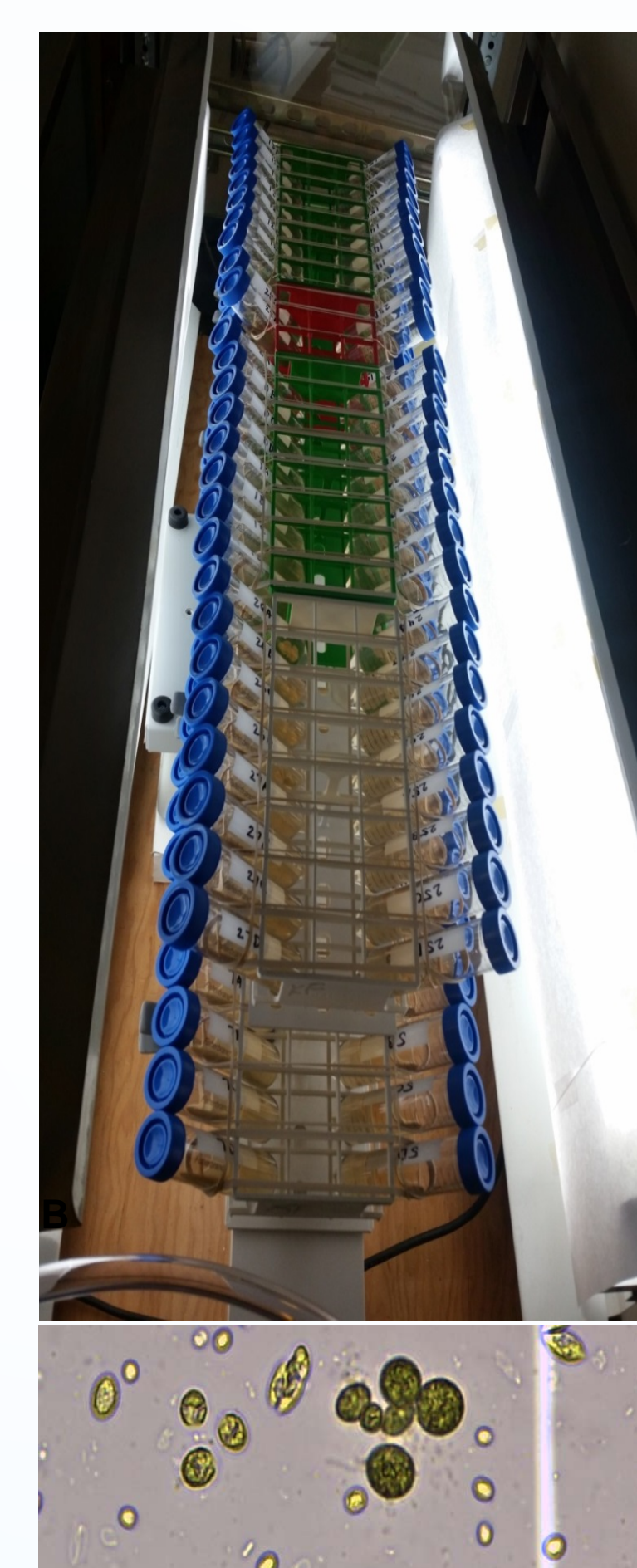


Figure 3. A) Photograph of high throughput algae-cyanobacterial polyculture experimental set up. B) Photographs of one of the top performing polycultures (treatment #10).

System Integration: DAIRIEES Modeling

DAIRIEES: Decision-support for Digester-Algae Integration for Improved Environmental and Economic Sustainability

- DAIRIEES is being used to track, quantify, and optimize C, N & P flows through the integrated system from manure to value-added co-products
- DAIRIEES is being configured to enable decision makers access to realistic and viable system operation, output and process yields, and techno-economic data in a format that ideally will enable data-informed decisions
- We are exploring opportunities to incorporate the DAIRIEES decision support tool in to the Innovation for U.S. Dairy's FARM-SMART™ tool box.

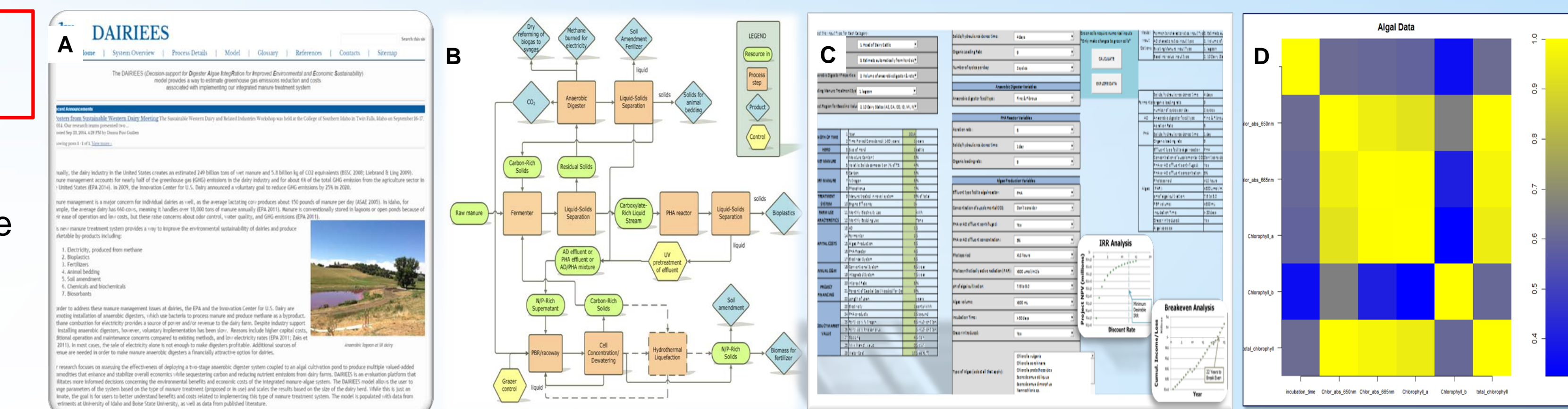


Figure 6. A) DAIRIEES webpage. B) DAIRIEES system flow model. C) DAIRIEES data input screen. D) DAIRIEES correlation analysis of AD-algal cultivation parameters.

Education and Outreach

- Our research team produced a short workshop for K-12 educators describing our integrated research program and provided an experimental framework and activity for K-12 educators to engage their students with.
- Currently, we are working with four 7th grade science teachers in Caldwell, ID and approximately 360 students to help address some of our algal diversity-productivity questions.

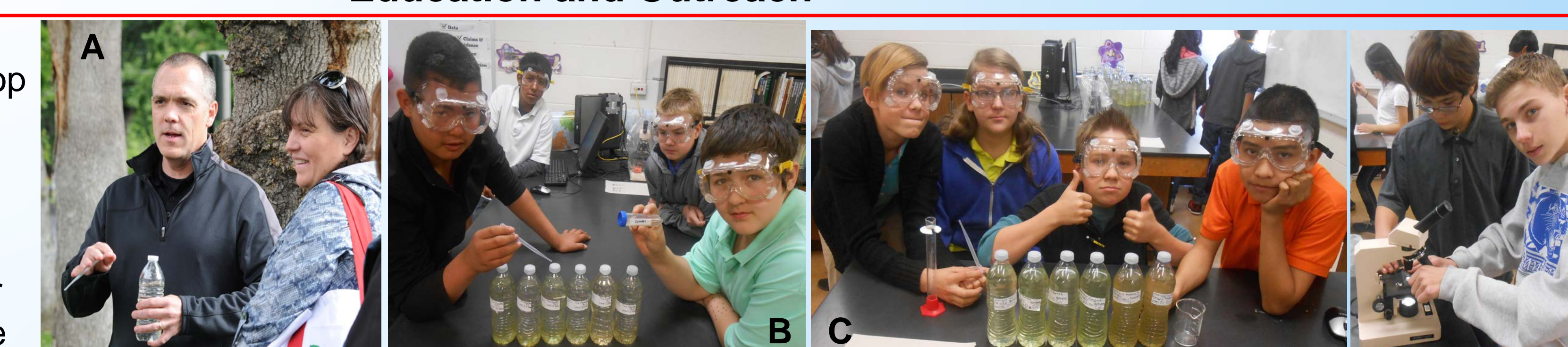


Figure 7. A) Erik Coats working with K-12 educators during 2014 iSTEM institute in Coeur d'Alene, ID. B - D) Young scientists from Syringa Middle School, 7th grade class. Students are setting up photobioreactors with a variety of algal species, dairy waste nutrients, and testing for which strains or polycultures produce the most biomass, and which strains/polycultures have the greatest resistance to rotifer grazing.

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