Biological Energy Institute
Clean Biofuels and Energy Independence

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Energy of the Present: Unsustainable Pollution

- Peak Oil
- Global Warming
- Economic Depression
- Political Conflicts

Oil versus Population Model
Energy of the Future: The Self-Sustaining Fuel System

- Increased Supply
- Sustainable Growth
- Conflict Aversion
- Improved Understanding

Biofuel versus Population Model
SIVE (Self-Sustainable System for Sustainable Energy)
Device Diagram: Overview
Systems-Level Overview

- Population Control
  - Self-regulation
    - Stabilize system as a whole
  - Cross-regulation
    - Algae and Bacteria codependent as a safety mechanism
  - External Factors
    - Self-regulation: Dependent on nutrient levels
    - Algae: Sunlight, ammonia, dissolved CO2
    - Bacteria: Dead algae, dissolved O2
- Nutrient Recycling
SIVE Device Diagram: A Closer Look

Organism Choices

• Algae
  o Modified B. Braunii
• Decomposer Bacteria
  o Why?
  o Putrefactors
  o Nitrosomonas
  o Nitrobacter

Container Choices

• Closed Photobioreactor
  o Experimental Design
  o Smaller, Cheaper, Controllable
SIVE Module Alpha: Feedback Control Overview

Diagram showing the flow of nutrients (CO2, N) and outputs (Oil, A_dead) through Algae.
SIVE Module Alpha: Feedback Control Transcription
<table>
<thead>
<tr>
<th>System Part</th>
<th>Description</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repressor Promoter</td>
<td>Allows for transcription of R.</td>
<td>Constitutively weak; strongly induced by X.</td>
</tr>
<tr>
<td>X Promoter</td>
<td>Allows for transcription of X.</td>
<td>Constitutively weak; strongly induced by Y, but repressed by R.</td>
</tr>
<tr>
<td>Terminator</td>
<td>Halts transcription of mRNA.</td>
<td>Necessary for inverter function.</td>
</tr>
<tr>
<td>Repressor Gene</td>
<td>Produces Repressor R protein.</td>
<td>Inhibits algae growth and X production, serving to decrease population levels; binds much better than Y to X Promoter.</td>
</tr>
<tr>
<td>X Gene</td>
<td>Produces X; likely accompanied by growth proteins.</td>
<td>Encourages algae growth; serves to temporarily increase population levels.</td>
</tr>
<tr>
<td>Y Protein</td>
<td>Allows for improved transcription of X Gene.</td>
<td>Produced by system bacteria; required for algae to produce X and survive.</td>
</tr>
<tr>
<td>External Factors</td>
<td>Likely Sunlight, Carbon Dioxide, and Water.</td>
<td>Establish an upper limit on algal growth, and therefore on bacterial growth.</td>
</tr>
</tbody>
</table>
SIVE Module Alpha Summary

• Method: Self-Regulation via Quorum Sensing
  - Basal X Expression
  - Inducer/Inverter Combination

• Motivation: Maximum Sustainable Fuel Output
  - Sustainability through Stability
  - Organism Escape Failsafe
SIVE Module Beta: Nutrient Recycling

- Sunlight
- Oil
- Algae
- Dead Algae
- Bacteria
- Nutrients [CO2, NO3, Y]
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</tr>
</thead>
<tbody>
<tr>
<td>Algae</td>
<td>Bioreactor workhorse. Decays into dead algae.</td>
<td>Produces oil.</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Bioreactor facilitator. Regenerates system nutrients.</td>
<td>Produces nutrients from dead algae and oxygen.</td>
</tr>
<tr>
<td>Sunlight</td>
<td>Provides energy to the system.</td>
<td>Fluctuating input.</td>
</tr>
<tr>
<td>Dead Algae</td>
<td>Decomposing algae; eventual nutrient source.</td>
<td>Intermediate energy component.</td>
</tr>
<tr>
<td>Nutrient Stream</td>
<td>Produced from dead algae by bacteria.</td>
<td>Consists of CO2, NO3, and Y protein.</td>
</tr>
<tr>
<td>Oil</td>
<td>Produced by living algae.</td>
<td>Overall system product.</td>
</tr>
</tbody>
</table>
SIVE: Testing and Debugging

- Self-Regulation Systems
  - Algae: Vary X levels, measure [algae]
    - Via signal, e.g. GFP and fluorescence levels
  - Bacteria: No self-regulation
- External Regulation Systems
  - Co-Regulation
    - Algae: Vary Y levels, measure [alg]
    - Bacteria: Vary X levels, measure [bac]
    - Test Alg and Bac together (w/o self-reg)
  - Nutrients
    - Alg and Bac: check that will grow indefinitely so long as nutrients sufficient
    - Ideally, sunlight: proportionally change alg rate constants
SIVE Modelling Equations

• \( \rightarrow \text{CO2} \)
• \( A + \text{NO}_3 + \text{CO2} + \text{Sunlight} + Y \rightarrow A + A + \text{Sunlight} + Y + O_2 + \text{CO2} \) (if have constant CO2 input)
• \( B + O_2 + A_{\text{dead}} \rightarrow B + B + \text{CO2}(\text{negligible}) + \text{NH}_4 \)
• \( B_2 + \text{NH}_4 \rightarrow B_2 + \text{NO}_2 \)
• \( B_3 + \text{NO}_2 \rightarrow B_3 + \text{NO}_3 \)
• \( A \rightarrow A + \text{Oil} \)
• \( B, B_2, X, Y \rightarrow 0 \)
• \( A \rightarrow A_{\text{dead}} \)
• Sunlight: probably want to cycle on off very rapidly (sin function)
• Initial inputs: Alg, Bac, NO3
• Constant Inputs: Sunlight, CO2?, misc. nutrients
• Assume well-mixed, no lag due to diffusion
If $X > T_{\text{high}}$:
    do not run $A \rightarrow A + A$
If $X < T_{\text{low}}$:
    do not run $A \rightarrow \text{oil} + A$
If $X < T_{\text{crash}}$:
    $A = 0$
If $Y > k_{\text{high}} \times X$
    do not run $B \rightarrow B + B$
If $Y > k_{\text{crash}} \times X$
    $B = 0$
SIVE Timing Diagram: Theoretical Optimum
Open Issues with SIVE

• Photobioreactor Design
  o Efficiency through Flow Loops (Light & Mixing)
  o Comparison with Static System
  o Issues of Algae/Bacteria Coexistence

• Additional Modules
  o Antibiotic Expression
  o Algae Protection / Flexibility

• Steady-State Rate Constants
Beyond Energy: SIVE Population Control in Biological Engineering

- Population Control in Biological Engineering
- Applications to Medicine & Bioremediation
- Foundational Experimental Tool
Decision
Sources

  Semi-closed Loop Algae-Diesel Fuel Photobioreactor Using Waste Water
  (03/04/2010, Innovative American Technology Inc. (Coconut Creek, FL, US)
- http://www.hielscher.com/ultrasonics/algae_reactor_cleaning_01.htm (mixing)
- http://pubs.acs.org/doi/full/10.1021/ie901459u
  Photobioreactor Design for Commercial Biofuel Production from Microalgae,
  Aditya M. Kunjapur, R. Bruce Eldridge, Industrial & Engineering Chemistry
  Research, (c) 2010
- http://partsregistry.org/Catalog
- http://www3.interscience.wiley.com/cgi-bin/fulltext/121634115/PDFSTART
- http://www.pnas.org/content/104/6/1877.full.pdf+html
"A high presence of oxygen around algae cells is undesirable. The combination of intense sunlight and high oxygen concentration results in photooxidative damage to algal cells.

Because of the constraint on the concentration of dissolved oxygen, tube length is limited in horizontal tubular reactors. This restriction makes it very difficult for tubular reactors to be scaled-up."

http://pubs.acs.org/doi/full/10.1021/ie901459u

These closed loop systems require many supplements to be added to provide nutrients for algae growth.