1. **Planned Activities:**
Data analysis as well as conducting additional co-culture experiments.

2. **Actual Accomplishments:**
During the last quarter, major effort has been on developing an unstructured model to describe the dynamic of the co-culture system, as well as comparing two genome-scale metabolic network models for *S. stipitis*. In addition, a new run of co-culture experiment was started.

**Modeling work:**
The initial dynamic co-culture model equations developed during last quarter were modified to fit the experimental data. Constraints were added on the fitted model parameters to ensure that the identified model makes physical sense. In addition, estimated model parameters were used to compare the specific rates of different operating conditions. In general, the identified model describes the experimental data well. However, current model cannot capture the oscillatory behavior observed in biomass concentration profiles of both strains under certain conditions. Literature search was conducted and we found multiple causes for the oscillatory behavior often observed in yeast strains under continuous culture conditions: product inhibition and cell heterogeneities are the two main reasons.

For metabolic network modeling, the main focus was analyzing two published genome scale metabolic network models, named model A and model B. First manual examination was performed, and several major discrepancies were identified. However, phenophase plane analysis shows that the two models are very similar. Due to the complexity involved in the genome-scale models, which consist of more than 1000 reactions, a more effective analyzing approach is needed. To address this difficulty, we proposed a system identification based approach to reveal the biological knowledge embedded in the metabolic network. Specifically, in the proposed approach, designed *in silico* experiments would be carried out first to perturb the network, then multivariate analysis would be carried out to analyze the data generated from the *in silico* experiments.
experiments to extract the underlying knowledge. With the proposed approach, we were able to conclude that Model B agrees with literature knowledge better.

**Experimental Work:**
Several difficulties related to equipment leakage were addressed, and a new run of co-culture pseudo-continuous fermentation was started successfully. In addition, a new design-of-experiment (DOE) for the co-culture experiment was conducted to reduce the time needed to run all cases for systematic investigation of the co-culture system dynamics.

3. **Explanation of Variance:** N/A

4. **Plans for Next Quarter:**
   We will further investigate the oscillation dynamics of our co-culture system and to modify the model according to the literature and new data set collected from the current co-culture experiment. We will also conduct additional co-culture experiments.

5. **Budget:**
   a. Funds Expended to Date (End of Reporting Period): $231,500
   b. Remaining Balance of Funds: $18,500

6. **Patents:** N/A

7. **Publications / Presentations:**


i) Liang M., Kim M.H., He Q.P. & Wang J. (2012), Impact of pseudo-continuous fermentation on the ethanol tolerance of *Scheffersomyces stipitis*, *Journal of Bioscience and Bioengineering*, under revision


l) Kim, M.H., Liang, M., He, Q.P. & Wang, J., Efficient bioconversion of glucose/xylose mixtures for ethanol production using a novel co-culture system, *AIChE annual meeting*, 2012, Pittsburgh, PA

1. **Name of Students Funded on Project:** including Department, Institution, Thesis/Dissertation Title (if applicable), Degree Obtained (if applicable), and Program Area:

<table>
<thead>
<tr>
<th>Student Name</th>
<th>Department</th>
<th>Institution</th>
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</thead>
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<tr>
<td>Liang, Meng</td>
<td>Chemical</td>
<td>Auburn</td>
<td>Experimental and in silico Fermentation of Glucose and Xylose with <em>Pichia stipitis</em></td>
</tr>
<tr>
<td>Kim, Min Hea</td>
<td>Chemical</td>
<td>Auburn</td>
<td>Kinetics of a Novel Co-culture System for Ethanol Production from Lignocellulosic Sugars</td>
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