ReadMe for S3 Publication

This ReadMe file directs users how to generate the Figures 1-5 shown in *Expedited screening of methanotroph-microalgae cocultures for integrated biogas valorization and wastewater remediation* (2025) by Loyal Murphy, Peter He, and Jin Wang. Each of the MATLAB Live Script files were developed in MATLAB R2023b, and it is strongly recommended that users run all codes in this GitHub in this MATLAB version. In addition, it is recommended that the user download the entire repository and keep the same file directories for maximum effectiveness of each code. Finally, please reach out to the corresponding author ([wang@auburn.edu](mailto:wang@auburn.edu)) if you have further questions regarding the GitHub folder.

For all each of the files, the number in front of the MATLAB code indicates which codes were used to generate the figures. In cases where there are two numbers associated with code, that Live Script contributes to both images in the publication. The one exception is “0\_Application,” as this was heavily utilized for the entire project but was not specifically used to generate plots. Below lists the following codes in numeric order.

“0\_Application” folder contains an application to facilitate selecting initial guesses for fitting their growth data to the parameters of a 4Z model. Running the application launches a GUI for the user to perform one of two actions. The user can either import data and adjust 4Z model parameters to fit their data visually or simply adjust Gompertz parameters without data to understand how each parameter changes the shape of the 4Z model. To play with the parameters without importing data, the user should select the “No Data” toggle and change one of the default 4Z parameters. There, they can change parameters to watch their plots change.

When importing data, the user will click the “Import Data” button. The user is prompted in MATLAB’s Command Window to select a .mat file with time and biomass measurements and specify how many replicates are used in the dataset. The data is then plotted on two separate plots. The first plot shows the biomass measurements over time. The second plot shows the approximate growth rate over time, using consecutive data points from the first plot. Each data point shows the exponential growth rate between consecutive data points at the average time of those consecutive data points. From here, the 4Z model fits both the biomass and growth rates over time are shown. The user can adjust each of the 4 parameters until they have fit the model of each growth plot to their liking. The user then clicks the “Export Data” button to export each of the 4 Gompertz parameters into the MATLAB workspace. This exported data can act as the initial guess for the non-linear solver used in the next parts.

“1\_Validation.mlx” contains the raw data and code used for Figure 1b. Running this code will generate this image. See “2\_Monocultures” for more information.

“2\_Monocultures.mlx” utilizes the raw data in the main directory of the folder to generate the entirety of Figure 2. This section is divided into methanotroph and microalga data, but the figures are combined at the end of the script for ease of reading. All methanotroph and microalga monoculture trial compiled data begins with the prefix “M” (methanotroph) and “P” (photoautotroph), respectively, followed by the shorthand for the methanotroph or microalga data. “2\_Monocultures” plots aggregate methanotroph data with 4Z fittings, followed by the results from the 4Z modeling protocol. Then, it plots aggregate microalga data with 4Z fittings, followed by the results from the 4Z modeling protocol. It calls the function “expo” which is included in the main directory; this function contains the piecewise exponential function described in the associated publication.

“3\_Cocultures.mlx” repeats the same procedure as “2\_Monocultures,” but it includes an initial section that corrects for the bias in initial biomass measurements (<0.25gDCW/L). In addition, each coculture plot is plotted with its respective monocultures to easily compare the differences Raw data for cocultures have a two-character prefix, the first character being the methanotroph species in the coculture and the second character being the microalga species. Cocultures containing *M. capsulatus* are marked with “C”; those containing *M. fibrata* are marked with “F”; and those containing *M. sp.* LW13 are marked with “L”. Similarly, cocultures containing *C. sorokiniana* are marked with “S”, and those containing *S. dimorphus* are marked with “D”. For example, all biomass data for the *M. fibrata* – *C. sorokiniana* coculture would be prefixed with “FS”. In addition, affiliated optical density data (wavelength = 750nm) and biomass measurements for all cocultures are labeled with “OD” or “gDCW” in their file names.

“3&4\_Mono\_v\_Coc.mlx” utilizes the monoculture and coculture results to compare growth data of the cocultures to their respective monocultures. It utilizes “f1.mat” – “f6.mat” to compile the data into a single image to generate Figure 3. This code also generates the plots used in Figure 4 but does not generate the figure directly. The user can change the axis labels at the bottom of the code to create both the full plots and the inset plots. Figure 4 was created by combining the results from this MATLAB code in Microsoft PowerPoint.

“5.1\_Temp\_Monocultures” contains the raw optical density data and code “0\_Cap\_Temps.mlx” that was used to capture the growth dynamics of *M. capsulatus* monocultures on diluted digestor effluent at different temperatures (30°C, 37°C, 42°C, 47°C). Here, the user can see how individual optical density wavelength scans were compiled into codes. This is different from the first four sections which show the consolidated versions of the raw data used for figure generation. The live script file separates the data in the folder based on the first 4 characters in the filename for each individual wavelength scan. In addition, the live script file uses the filename to extract time data down to the second the scan was taken. From there, the user selects the wavelength desired for optical density to gram dry cell weight (gDCW) conversion. For this publication, the authors selected a wavelength of 600nm, and using linear interpolation found that gDCW*M.cap* = 0.235\*OD600. The live script isolates all wavelength scan data at 600nm and plots the gDCW based on the linear relationship. Lastly, the code generates “f” .mat files to carry data over to the next Live script file in the “5.2\_Temp\_Cocultures” folder.

The final folder is “5.2\_Temp\_Cocultures.” This is like “5.1\_Temp\_Monocultures” except it contains all the *M. capsulatus* coculture data at 30°C and 37°C. The live script file to generate the plots can be found as “0\_Cap\_Cocultures.mlx.” All the file names and live script explanation follow the same convention as described in the paragraph explaining “3\_Cocultures.mlx”. It utilizes the “f” .mat files generated from the monoculture data to combine into a single figure. Running the codes in “5.1\_Temp\_Monocultures” followed by “5.2\_Temp\_Cocultures” will generate Figure 5.

This concludes the overarching explanation for this GitHub folder associated with *Expedited screening of methanotroph-microalgae cocultures for integrated biogas valorization and wastewater remediation* (2025) by Loyal Murphy, Peter He, and Jin Wang. The authors highly encourage users to implement the 4Z model for their growth data to extract biologically relevant information. Feel free to let the authors know that you are implementing the code into your research and cite the publication to show your support. Check out the author’s associated methodology paper – *A Modified Gompertz Model and Its MATLAB Implementation for Microbial Growth Performance Assessment* (MethodsX 2025) – for a more detailed explanation of applying the model.

Again, feel free to reach out to the corresponding author ([wang@auburn.edu](mailto:wang@auburn.edu)) if you have any comments, questions, or suggestions for model improvements!