5 Discussion

5.1 Summary of Results

The calibration data analysis indicated that the models are prone to under-predicting the bacterial concentration of the phycosphere modeling system which is both an effect of the true concentration of bacteria and Chlamydomonas during any absorbance measurement. Specifically, as the true concentration of bacteria increases, so too does the severity at which the true Chlamydomonas content impacts the predicted values of the bacteria in the system. Therefore, the bacterial content cannot be determined without the need to correct them which can only be done post-prediction. Fortunately, the models are able to capture, with a high degree of accuracy, the content of Chlamydomonas at low concentrations of bacteria ($A_{560} < 0.4$). Since the Beer-Lambert models predict Chlamydomonas content well, it is possible to correct the predicted bacterial concentrations.

The results also indicate the extent of over and under-predictions for any true concentration of bacteria and Chlamydomonas, predictions are model dependent. Since the models can only be created which make use of the measured absorbance signals, the extent of these discrepancies are variable. Models which have K values approaching zero tend to perform worse than those which have a higher K values, as determined in Results & Analysis 4.3. With an absorption spectra that covers the breadth of visible light, a brute force determination of an optimal model can be determined more accurately. The calibration data used for this thesis indicate that optimal model results when the wavelengths 540, 680 and 750nm are measured, but point to the existence of more optimal model. When said wavelengths are measured and subsequently modeled, the observation of the inter-component concentration dependencies (Chlamydomonas effect) allow for correction factor models to be constructed. Application of the resulting correction factors on the calibration data show a significant increase in model performance.

The models and their subsequent correction factors, however, remain unvalidated. Therefore, it is not possible to answer the main question originally given at the onset of this project;

which bacterial strains impart beneficial or detrimental effects on their host, Chlamydomonas, with any degree of accuracy. That this question remains unanswered is not only the result of the unvalidated models, however, as the data given to answer this question had low empirical absorbance measurements which can only yield predictions which fall below the limit of detection of the Tecan.

The results of the model transferability experiments, from the Tecan-measured calibration data to the photobioreactor (PBR) was somewhat more successful. The results for this experiment were largely in accordance to the expected values and suggest that given properly corresponding wavelength measurements the models will predict the PBR's measurement as they would be predicted with Tecan-measured data. Consequently, as the models correction factors remain unvalidated the predicted values for the PBR experiment data cannot be analyzed with with any degree accuracy. Nevertheless, these models and corrections were applied and indicate that the viability of this method of monitoring the system should be feasible. However, more data is necessary to examine whether the Chlamydomonas effect has the same proportion as observed in the Tecan-measured calibration data given the phycosphere modeling vessels have larger volumes than the microplate wells.

Photobioreactor measurements and its sample's 16S profile relative abundances were then compared to the corrected prediction-cell count conversion's relative abundances. The results suggest that the relative abundances, as measured by 16S profiles, of Chlamydomonas are far higher than what the cell count capture. Literature review details that the 16S sequences of Chlamydomonas are far higher in abundance per unit cell than those of bacteria, therefore special conversations are necessary to bring these results to proper accordance.

5.2 Implications

The determination of K-Space (Section 4.3) suggest the existence of a better performing model (Model S) when its predictions are corrected by the correction factors. This means that the currently measured wavelengths would need to be changed to match the wavelengths required by Model S. Furthermore, since the predictions yield an absorbance value at a currently unmeasured wavelength (750nm), the predicted results cannot be compared to that same empirically measured wavelength, which is a requirement to uphold the Beer-Lambert law (Section 4.1.2, Figure 4.4). However, without the validation of the correction factors, it cannot be stated with any degree of certainty if the models and their predictions can be

applied to any of the photobioreactor derived data in the manner envisioned in the research group.

5.3 Limitations

The constraints imposed by currently measured wavelengths do not allow for Model S or any other better performing model, yet to be determined in K-Space, to be leveraged on the data. Preliminary results indicate that the current models also have a correction factor, although, with less performance that the corrections of the predictions of Model S. With more data, it may be possible that these limitations can be overcome on the currently measured wavelengths, however, these correction factors will need to be validated as well. The possibility that the Chlamydomonas effect is different in the photobioreactor remains to be verified as well, and without Tecan-measured PBR samples the extent of the difference of this effect between them cannot be corrected.

5.4. Recommendations

As determined by existence of better performing models residing in K-Space, it is recommended that a new and broader K-Space be determined. This would yield the best predictive model which correction factors, once validated, would predict values with the type of accuracy necessary to optimally enhance the phycosphere model monitoring system. This dictates however, that the PBR be fitted to measure those wavelengths, as the currently measured ones have a smaller range of possible corrections. Furthermore, it may be that the predictions and corrections are dependant on the manner in which the calibration data is composed, with respect to the concentrations of the individual strains that make up any SynCom. Therefore, several bacterial concentrations are required in order to verify that the Chlamydomonas effect is not dependant on their individual concentrations. Consequently, this would also mean that a calibration be made for every SynCom designed.