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UNIVERSITY OF CALIFORNIA SAN DIEGO

Nitrogen-source-specific utilization of biodegradable plastic monomers by heterotrophic marine
bacteria

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Marine Biology

by

Diego Garcia

Committee in charge:

Professor Katherine Barbeau, Chair

Professor Andrew Allen

Professor Lihini Aluwihare

2026

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University of California San Diego

2026

EPIGRAPH

Have you entered into the springs of the sea
Or walked in the recesses of the deep?
Have the gates of death been revealed to you,
Or have you seen the gates of deep darkness?
Have you understood the expanse of the earth?
Tell *Me*, if you know all this.

Job 38:16-18

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LIST OF ABBREVIATIONS

3HB	3-Hydroxybutyrate
PHB	Polyhydroxybutyrate
INT	2-Para (iodo-phenyl)-3(nitrophenyl)-5(phenyl) Tetrazolium Chloride
POC	Particulate Organic Carbon
DIN	Dissolved Inorganic Nitrogen
ROS	Reactive Oxygen Species
ETS	Electron Transport System
CAC	Citric Acid Cycle
OD ₆₀₀	Optical Density at 600 nm
ANOVA	Analysis of Variance Test
BGE _E	Estimated Bacterial Growth Efficiency

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VITA

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ABSTRACT OF THE THESIS

Nitrogen-source-specific utilization of biodegradable plastic monomers by heterotrophic marine
bacteria

by

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Master of Science in Marine Biology

University of California San Diego, 2026

Professor Katherine Barbeau, Chair

In response to the accumulation of plastic waste in the environment, the development of degradable bioplastics as an alternative has been highly sought after, yet should certain bioplastics succeed the non-biodegradable plastics, there may be implications of how the accumulation of this more bioavailable form of plastic impact the carbon cycle, particularly in

the marine environment, where nutrient conditions are diverse and play an important biological role in carbon utilization. This Thesis examines the model marine bacterium *Alteromonas macleodii* ATCC 27126's ability to utilize 3-Hydroxybutyrate (3HB), a monomer of the bioplastic Polyhydroxybutyrate (PHB) among glucose, galactose, and maltose, components of natural polysaccharides and certain bioplastics, depending on the provision of reduced nitrogen (NH_4) or oxidized (NO_3). According to Optical Density (OD_{600}) growth curves, cell counts, and respiration determined by the INT Reduction method, 3HB resulted in peak biomass levels comparable to the sugars, but led to slower growth and was less efficiently incorporated into biomass; this may imply that at a metabolic level, PHB, or organic acid-based bioplastics, is more recalcitrant and may sink as Particulate Organic Carbon (POC), but when degraded, less PHB-derived biomass would sink as POC. With the more bioavailable NH_4 as the sole nitrogen source, 3HB resulted in higher peak levels of biomass and a marginally higher growth efficiency when compared to NO_3 , yet the nitrogen-source preference was flipped towards NO_3 with the sugars. These results add nuance to designing replete conditions in laboratory studies, as more bioavailable substrates do not innately result in better growth.

INTRODUCTION

With over 400 million tons of plastic being globally produced each year, plastic has become a problematic and yet unescapable element of today's modern global economy (UNEP, 2021). Much of the plastic is used in food and pharmaceutical packaging as an energy and cost-efficient method for keeping products free from bacterial contamination, in medical single-use products intended to prevent infections, and synthetic textiles fibers to manufacture clothing and furniture (Benson et al., 2021; Evode et al., 2021; The Fiber Year, 2017). Most plastics, whether intentionally or not, have brief lifespans and are rapidly discarded, as of 2015, only 30% of plastic products ever produced were still in use; plastic waste generated is projected to reach over 25,000 million metric tons (Mt) by 2050. Due to littering and poor waste management, much plastic waste is leaked into the ocean, with 11 Mt entering annually; accounting for a global increase in plastic production, this value is projected to triple around 2040 (Geyer et al., 2017). Floating plastic debris tends to entangle, be ingested by, or lacerate marine tetrapods, fish, and arthropods, reducing their survivability and fitness (Laist, 1997). Plastic is recalcitrant to biodegradation, and is usually not able to be broken down by microorganisms to be utilized as a carbon substrate, but is only effectively broken down through mechanical or photo-degradational means as microplastics persisting in the environment (Gewert et al., 2015; Koelmans et al., 2015). For the waste that is managed, most plastic is landfilled, where it will not effectively decompose, and the rest is either incinerated or recycled, processes which both release microplastics and toxic additives into the environment to varying degrees (Nafiu et al., 2025; UN, 2023; D. Wu et al., 2021; Yang et al., 2021). Due to the apparent failure of plastic waste management, there have been efforts to consider alternative forms of plastics, bioplastics, that may avoid these consequences.

Bioplastics is a term that generally refers to materials with similar properties to conventional plastics, but are derived from biological materials and/or processes, and may or may not be decomposed through biological means, but for this Thesis, “bioplastics” will only be used to refer to plastic-like materials that are also biodegradable. Bioplastics are biodegradable due to their polymer chains containing the ester and amide bonds that microorganisms, such as bacteria and fungi, can enzymatically break apart, hydrolyzing the plastic into smaller pieces. Fully biodegradable plastics feature repeating units in their polymer chains, monomers, that microorganisms can intake as a carbon substrate and convert into biomass, energy, water, and CO₂, as any other molecule of organic matter would decompose. As a biological process, decomposition rate is dependent on not only the structure of bioplastics, but also the conditions of the environment, such as the size and community of the decomposing microorganisms, temperature, and oxygen availability (Lott et al., 2021; Zhang et al., 2025). Due to most bioplastics containing virtually no nitrogen, the biodegradation rate is limited by nitrogen which is required mainly for upregulating enzymes to break apart the polymer bonds and nucleic acids for biomass production (Guliyev et al., 2023; Reay et al., 2024).

Ideal conditions for decomposition are not common in nature, but present in man-made composters. However, due to the increasing amounts of plastic waste leaking into the environment, it is important to consider how bioplastics, given that they are managed similarly to conventional plastics, would biodegrade in these natural environments, such as in the ocean. Due to the low temperatures and limited oxygen in many marine environments, decomposition of bioplastics in the ocean is expected to occur at low rates, for example, Polylactic Acid (PLA) does not degrade in aquatic environments (Nazareth et al., 2019; Ribba et al., 2022; Royer et al., 2023). Despite the demand for characterizing the degradation rate of bioplastics, the method to

do such requires studies to conduct incubation experiments on the timescale of months to observe significant degradation, if the plastic is biodegradable within that frame (Dilkes-Hoffman et al., 2019). This Thesis explores using the monomer sub-units of bioplastics as a carbon substrate and studying how the monomer is metabolized, shortening the incubation timescale to weeks or less. Our main goal is to compare the metabolism of carbon substrates used in bioplastics, along with discerning the nutrient regimes most appropriate for their utilization and discussing possible implications on marine bioplastic degradation in the ocean carbon cycle. There seem to be few studies that investigate these aspects of biodegradation (Guliyev et al., 2023; Reay et al., 2024; Sanz-Lázaro et al., 2021; Thellen et al., 2008; Zhao & Zhu, 2025; Ziervogel et al., 2024).

Respiration is central to assessing the carbon flux direction in waters, as organic matter produced by CO₂-fixing photosynthesizers is either exported to the ocean floor as Particulate Organic Carbon (POC), or is remineralized back into gas, particularly by heterotrophic marine bacteria, and studies would assess particular waters as “net heterotrophic” if more POC is respired than produced (del Giorgio & Duarte, 2002). However, biodegradable plastic deposition in the ocean represents an allochthonous carbon input uncoupled from primary production that can produce its own bacterial activity to be taken into account (Zhao & Zhu, 2025). One study found that the deposition of biodegradable plastics in sediments, due to being nitrogen-poor, stimulated the sediment community to degrade existing buried organic matter and acquire nitrogen (Sanz-Lázaro et al., 2021). Nitrogen in the form of Dissolved Inorganic Nitrogen (DIN), which includes NH₄ and NO₃, is a crucial factor in organic matter cycling, for waters limited by DIN lead to the accumulation of Dissolved Organic Matter (DOM) (Hale et al., 2017). This element is necessary for the amino acids necessary to form the enzymes that break

down carbon and form biomass, along with participating in bacterial replication, incorporated into the nucleotides that build DNA. Despite this, there is still a dearth of studies that examine the implications of the bacterial respiration of plastic-derived carbon on marine carbon flux, as in, whether more labile plastics are more likely to be exported as POC than contribute to the dissolved CO₂ pool, as high bioavailability does not necessitate high biomass incorporation (del Giorgio & Cole, 1998).

3-Hydroxybutyrate (3HB) is the organic acid monomer of the bioplastic Polyhydroxybutyrate (PHB), which is most widely applied in medical implants and pharmaceuticals and is likely to be degradable by marine bacteria, as the marine community is generally considered to be synthesizers of PHB; though, degradation rates are found to be slow in marine environments, copolymer variants demonstrate faster degradation (Bagheri et al., 2017; Dilkes-Hoffman et al., 2019; Nanda et al., 2022; Ray & Kalia, 2017; Santos et al., 2017; Thellen et al., 2008). Glucose is a very significant metabolic precursor for energy and biomass production and is widely considered to be representative of the marine labile DOM pool (Blanco & Blanco, 2022; C. A. Carlson & Hansell, 2015; Hale et al., 2017; Mopper et al., 1980; Rich et al., 1997). This sugar is not only complexed in natural polymers such as glycogen from animals, starch and cellulose from plants, and laminarin from algae, but is also used in bioplastic technologies (Alcázar-Alay & Meireles, 2015; Marinho, 2025; Neoh et al., 2024; Qasim et al., 2021; Wang et al., 2026). In contrast to 3HB, glucose represents more degradable polymers (Arnosti, 2011; Ghobish et al., 2025). Galactose is a structural isomer of glucose that comprises a significant fraction of the dissolved sugar pool and is complexed in natural polymers such as fucoidan and agarose from algae (Goldberg et al., 2009; Wang et al., 2026). Maltose is not a monomer, but a disaccharide of two glucose molecules linked by a glycosidic bond prevalent in

glycogen and starch; its utilization relative to glucose may be used to extrapolate how larger polysaccharide complexes would be degraded. The four substrates: 3HB, glucose, galactose, and maltose, and the effects of nutrient conditions will be experimentally determined between fully oxidized nitrogen, NO_3 and fully reduced nitrogen, NH_4 , by measuring growth over time and oxygen consumption of each nutrient regime. The bacterium used for these incubations is *A. macleodii*, ATCC 27126, a widespread marine heterotroph that grows very quickly in controlled laboratory settings and is representative of a diverse consortium of plastic degrading organisms through its large genome that allows for the utilization of a diverse array of carbon substrates (Baumann et al., 1972; López-Pérez et al., 2012; Pedler et al., 2014; Wietz et al., 2022).

MATERIALS AND METHODS

Experimental Growth Conditions

A. macleodii ATCC 27126 was plated on Marine Broth 2216 agar from preserved, frozen glycerol stocks to obtain isolated colonies. Before experimental incubation, a colony from the agar plate was inoculated into 10mL PC+ cultures and grown on a Lab-Line Environ Orbital Shaker 3528 - Analog at ~190 RPM overnight to condition the bacterium into peak growth state. PC+ media was prepared according to the supplementary protocol used for studying *A. macleodii* (Manck et al., 2022). The carbon and nitrogen concentrations in this recipe reflect typical replete marine bacterial media for copiotrophs (Goto et al., 2017; Kimes et al., 2014; Manck et al., 2020). Minimal carbon media was prepared similarly to the PC+ media, excluding casein and bacteriological peptone to control growth to sole sources of carbon and nitrogen, and the use of Chelex® 100 resin, for iron contamination was not a concern for this Thesis. Minimal carbon stock media at equal experimental concentrations of 0.02 M were prepared for each carbon source used: 3HB, glucose, galactose, and maltose. All mediums were buffered to pH ~7.8 with NaOH and HCl solutions. After pre-experimental incubation in PC+ media, 100 µL of bacterial volume were each inoculated into 10 mL volumes of minimal carbon media, along with 1:1000 aliquots of the trace metal mix, vitamin mix, KH₂PO₄, FeCl₃, and Ethylenediaminetetraacetic acid (EDTA) solutions also used in (Manck et al., 2022). To experimentally vary the nitrogen regimes, 0.94 M of filtered NH₄Cl and 0.94 M of filtered NaNO₃ were each prepared in Milli-Q H₂O (MQ) and added as 1:20 aliquots along with the other nutrients; only one nitrogen source was added to each culture, to get final nitrogen concentrations of 4.7 mM. Each carbon and nitrogen regime incubation was performed in triplicate unless otherwise stated.

Table 1. All 8 Experimental Growth Regimes studied, categorized by Carbon source, Nitrogen source, and abbreviation used to refer to each regime.

Carbon	Nitrogen	Abbreviation
3HB	NH ₄	3HB-A
3HB	NO ₃	3HB-N
Glucose	NH ₄	Glu-A
Glucose	NO ₃	Glu-N
Galactose	NH ₄	Gal-A
Galactose	NO ₃	Gal-N
Maltose	NH ₄	Glu-A
Maltose	NO ₃	Glu-N

Optical Density Growth Curve

Each culture was monitored at least once every ~24 hours using a Genesys™ 20 UV-VIS Spectrophotometer (Thermo Scientific) measuring the Optical Density at 600 nm (OD₆₀₀) of 0.5 mL aliquots of culture volume. The cultures were grown until the first negative change in culture volume OD₆₀₀. The carrying capacity (K) of each culture was assumed to be the peak OD₆₀₀ value, and R's growth rate package was used to fit a logistic growth model and calculate the max specific growth rate ($\mu_{\max}$) (Manck et al., 2022). To check for effects of equal carbon concentrations of substrate in the case of 3HB (4 C) and maltose (12 C) in comparison to glucose and galactose (6 C), OD₆₀₀ growth curves at 1.5x and 0.5x original concentration, respectively, were also produced.

Flow Cytometry Cell Counts

Cultures were grown until near carrying capacity as predicted by OD₆₀₀ growth curves, which differed depending on nutrient regime (24 hrs for maltose, 48 for glucose and galactose, 96 hrs for 3HB-A, and 144 hrs for 3HB-N); cultures were vented daily in a sterile environment to control for artifacts potentially introduced by withdrawing aliquots in the OD₆₀₀ growth curve cultures. 1 mL aliquots of culture volume were filtered through a 40 µm mesh cell strainer rinsed with MQ and into a 1.8 mL cryovial. 0.2 µm filtered glutaraldehyde was added to the cryovial to make a 0.5% dilution; the cryovial was capped, gently shaken and incubated at 2 °C for ~10 min. After the culture volumes were fixed, the cryovials were stored at - 80 °C until further analysis. The cell densities of the culture volume samples were measured using a Guava® easyCyte™ Flow Cytometer (Cytex). Following the cleaning and calibration of the easyCyte, 96-Well, Round Bottom, Non-Treated Assay plates (Corning™) were prepared with 200 µL dilutions of 1:200 sample volume in autoclaved 0.2 µm filtered Scripps Pier Seawater in technical triplicate. Each well, including 0.5% glutaraldehyde seawater blanks, was inoculated with 2 µL of 100X SYBR™ Green (Thermo Fisher) in 0.2 µm filtered DMSO (diluted from 10,000X). The plates were incubated and shielded from light in a Multi-Therm™ Shaker (Benchmark) at 350 rpm for 30 min, before the samples could be read by the easyCyte. Cells were counted and distinguished from cell fragments and other associated artifacts of the preparation method by exciting with a 488 nm light and plotting green fluorescence hyper-logged (GRN B Hlog) on the x-axis and red fluorescence hyper-logged (RED B Hlog) on the y-axis and gating under the 1:1 noise line from 10² to 10⁵ GRN B Hlog.

Short Term Oxygen Consumption Assay

The oxygen consumption of *A. macleodii* was determined via a colorimetric indicator, 2-para (iodo-phenyl)-3(nitrophenyl)-5(phenyl) tetrazolium chloride (INT), a salt which is reduced by the first three complexes of the Electron Transport System (ETS) in cells, into a water-insoluble precipitate, iodonitrotetrazolium violet-formazan (INT-F); this precipitate scales with the abundance of respiratory enzymes, and when dissolved and read on a spectrophotometer, can be a robust indicator of ETS activity, and therefore O₂ consumption during the short-term assay (Martínez-García et al., 2009; Packard, 1971; Smith & McFeters, 1997).

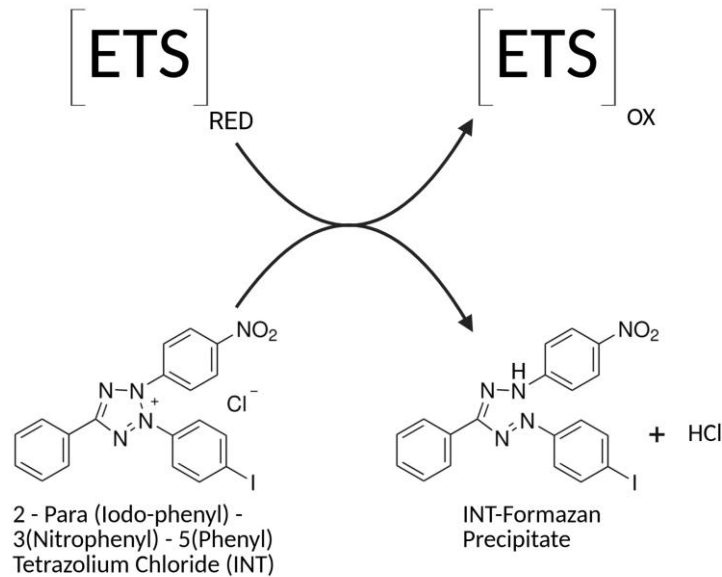


Figure 1. Oxidation reaction between INT and ETS complexes; adapted from (Packard, 1971); illustration created with BioRender.

Along with live triplicate cultures, a fourth culture is grown to serve as a blank and capture any abiotic reduction of INT. Cultures were grown until near carrying capacity as predicted by OD₆₀₀ growth curves. Formalin (FA) solution was added to the blank to make a 2% dilution, and after 15 min of fixing, all cultures were inoculated with 7.9 mM solution of INT in 0.2 µm filtered MQ to produce a final INT concentration of 0.2 mM. The assayed cultures were

incubated in the dark on the shaker at ~190 RPM for 2 hours; highly productive systems were recommended not to be incubated for longer than 2 hours due to deviations from linear INT-F precipitation as a result of INT toxicity or senescence. After incubation, all the live cultures were fixed with FA for 15 min. All the fixed cultures were passed through a vacuum filtration system, drying the precipitate onto 0.2 µm Polystyrene (PS) filters, to be stored in tubes and stored at 2°C until the day of analysis.

On the day of analysis, the samples were dissolved in 10 mL of 2-propanol, sonicated at 50°C in an FS20H 42 Hz Sonicator (Thermo Fisher) for 25 min, and vortexed for 15 sec. Subsequently, 500 µL of the extract was further diluted 1:1 with 2-propanol in a 1 mL microcentrifuge tube and centrifuged at 13,000g in a Fresco™ Microcentrifuge (Sorvall) for 10 min. Samples that did not appear to precipitate much INT-F were dissolved in only 5 mL of 2-propanol to increase sensitivity. Absorbances of centrifuged extracts were measured at 485 nm in a Cary Series UV-Vis Spectrophotometer (Agilent). 9 INT-F standards from 0.1 µM—100 µM were prepared to develop a linear calibration curve comparing concentration with absorbance at 485 nm. The INT-F concentration of each culture, deriving only from the reduction of INT can be used as an indicator of O₂ consumption via this linear relationship pre-calibrated by a recent study investigating the respiration of prokaryotic copiotrophs (Seguro et al., 2025):

$$\log(O_2) = 0.36 * \log(INT - F) + 1.21 \quad \text{Eq. 1}$$

where O₂ is the consumption rate of O₂ and INT-F is the precipitation rate of INT-F during incubation, both in units µM/hr.

Bacterial Growth Efficiency Estimation

An estimation of Bacterial Growth Efficiency (BGE_E) for each culture nutrient regime was calculated from this equation:

$$BGE_E = \frac{BP_E}{BP_E + BR_E} \quad \text{Eq. 2}$$

where BP_E is estimated Bacterial Production and BR_E is estimated Bacterial Respiration, both in units $\mu\text{g C}$ (del Giorgio & Cole, 1998). BP_E was roughly estimated with Cell Counts by assuming a constant Per-Cell Carbon Biomass of $141.816 \pm 32.937 \text{ fg C}$ and using the equation:

$$BP_E = C_{cell} * N \quad \text{Eq. 3}$$

where C_{cell} is the Per-Cell Carbon Biomass in ug/cell and N is the total enumerated cells/L (Fouilland et al., 2018). The Per-Cell Carbon Biomass value was obtained by multiplying the C quota with the Biovolume of an Oceanic Strain of *Alteromonas* under Replete conditions; the error was propagated from the triplicate standard deviations reported in the study (Fourquez et al., 2014). BR_E was roughly estimated with the short-term O_2 Consumption by using the logistic growth model from the OD_{600} curves and integrating to estimate total O_2 consumption, using the equations:

$$OD(t) = \frac{K}{1 + \left(\frac{K-0.015}{0.015}\right) * e^{-\mu t}} \quad \text{Eq. 4}$$

$$O_{Total} = \int_0^T O_2 * \frac{OD(t)}{K} dt \quad \text{Eq. 5}$$

where K is the model's carrying capacity in OD_{600} , μ is the model's max specific growth rate in hr^{-1} , OD is the model's Optical Density at time t , T is the growth time in hr , and O_{Total} is the total consumed O_2 in μM . O_{Total} was converted to BR_E by assuming a constant substrate-specific Respiratory Quotient and using the equation:

$$BR_E = O_{Total} * RQ * 12 \quad \text{Eq. 6}$$

where RQ is the Respiratory Quotient in mol CO₂/mol O₂ (Del Giorgio & Williams, 2005; Martínez-García et al., 2010; Posacka et al., 2019). The RQ for 3HB was assumed to be 0.89, and the RQ for the sugars was assumed to be 1 (Berggren et al., 2012; Frayn, 1983).

Statistical Analysis

We used two-way Analysis of Variance Tests (ANOVA) for the significant effects of multiple variables: carbon sources, nitrogen sources, and their interactions. Data was log-transformed to improve normality of residuals. We also conducted post hoc analysis using Estimated Marginal Means (EMM) to evaluate pairwise significant differences between the effects of each experimental nutrient regime, with a Tukey's Honest Significant Difference test (HSD) to adjust for multiple family comparisons. The model residuals were assessed for ANOVA compatibility using Shapiro-Wilk tests and Quantile-Quantile (Q-Q) plots for Residual Normality and Levene's test for Homoscedasticity. Data that departs moderately from normality are assessed to be still compatible with ANOVA due to its relative insensitivity to non-normality (Box, 1953). The significance thresholds of $\alpha = 0.05$, 0.005, and 0.001, being borderline significant, significant, and highly significant, respectively, were used for all tests (Chambers and Hastie, 1992; R Core Team, 2021). All plots were prepared using R with ggplot2, patchwork, and magick (Ooms, 2026; Pederson, 2025; Wickham, 2016).

Metabolic Pathway Investigation

Pathways described and discussed herein are hypothesized from a framework of *Alteromonas macleodii* ATCC 27126's BioCyc Database, developed through synthesis of predicted computational inference from the strain's genome and manual curation with evidence from experimental studies, and supplemented with speculation from related organisms to fill

gaps in knowledge on the model organism (Karp et al., 2019; López-Pérez et al., 2012; Sher et al., 2025)

RESULTS

Table 2. All statistics determined in this Thesis: Max Specific Growth Rate ($\mu_{\max}$), Carrying Capacity (K), Cell Counts (N), Short Term O₂ Consumption, and Estimated Bacterial Growth Efficiency (BGE_E); associated errors are standard deviations from biological triplicates or propagated error in the case of BGE_E.

Nutrient Regime	$\mu_{\max}$ (hr ⁻¹)	K (OD)	N (cells/mL)	O ₂ Consumption (μM/hr)	BGE _E
3HB - A	0.093 ±0.006	0.734 ±0.005	17.96*10 ⁶ ±15.78*10 ⁶	57.67 ±2.53	0.071 ±0.060
3HB - N	0.039 ±0.002	0.606 ±0.037	54.64*10 ⁵ ±29.56*10 ⁵	45.51 ±1.86	0.029 ±0.017
Glu - A	0.432 ±0.021	0.170 ±0.003	44.34*10 ⁵ ±17.23*10 ⁵	-	-
Glu - N	0.294 ±0.031	1.160 ±0.038	20.27*10 ⁶ ±1.98*10 ⁶	52.40 ±2.30	0.121 ±0.027
Gal - A	0.281 ±0.018	1.010 ±0.018	17.07*10 ⁶ ±4.90*10 ⁶	48.45 ±0.86	0.112 ±0.037
Gal - N	0.660 ±0.009	1.200 ±0.035	36.05*10 ⁶ ±7.69*10 ⁶	50.29 ±5.15	0.170 ±0.047
Mal - A	0.575 ±0.098	0.293 ±0.007	72.82*10 ⁵ ±24.92*10 ⁵	5.87 ±0.98	0.438 ±0.110
Mal - N	0.598 ±0.008	0.620 ±0.006	18.05*10 ⁶ ±7.17*10 ⁶	9.64 ±1.93	0.555 ±0.124

Growth Time-Series

A visual demonstration comparing the OD₆₀₀ growth curves and statistics of *A. macleodii* ATCC 27126 on each experimental nutrient regime is displayed in (Fig. 2). Glu-N and galactose induced the highest carrying capacities, 3HB and Mal-N induced medium carrying capacities, and Gluc-A and Mal-A induced the lowest carrying capacities. 3HB induced the lowest growth

rates and Gal-N, Mal, and Glu-A induced the highest growth rates, yet Mal-N dropped to nearly 50% of its carrying capacity after 24 hours.

Shapiro-Wilk Test for Normal Residuals passed for $\mu_{\max}$ and K ($p = 0.353$, $p = 0.753$), Leven's Test for Homoscedacity passed for both statistics ($p = 0.478$, $p = 0.487$), and Q-Q plots for both statistics demonstrated approximate residual normality with slight deviations; these diagnostic tests indicate that this data set is appropriate for ANOVA analysis and yet is not without flaw. ANOVA analysis demonstrated a highly significant effect of carbon source and of interactions ($p < 0.0001$) on $\mu_{\max}$ and highly significant effects of carbon source, nitrogen source, and their interactions ($p < 0.0001$) on K.

A visual demonstration comparing the significant differences among the growth statistics is displayed in (Fig. 3). The first EMMS pairwise contrast demonstrated a highly significant negative effect of NO_3 with 3HB and galactose ($p = < 0.0001$) and a significant negative effect of NO_3 with glucose ($p = 0.0027$) on $\mu_{\max}$, and a borderline significant positive effect of NO_3 with galactose ($p = 0.0261$) and highly significant positive effects of NO_3 with glucose and maltose ($p < 0.0001$) and a negative effect with 3HB ($p = 0.0003$) on K.

The second pairwise contrast demonstrated a highly significant positive effect ranking of maltose, to glucose, and to galactose, over 3HB with NH_4 ($p < 0.0001$) and highly significant positive effect ranking of galactose, to maltose, and to glucose, over 3HB with NO_3 ($p < 0.0001$) for $\mu_{\max}$, a highly significant positive effect ranking of galactose, to 3HB, and to maltose, over glucose with NH_4 ($p < 0.0001$) and a highly significant positive effect ranking of glucose to galactose over 3HB with NO_3 and also over maltose with NO_3 ($p < 0.0001$) on K.

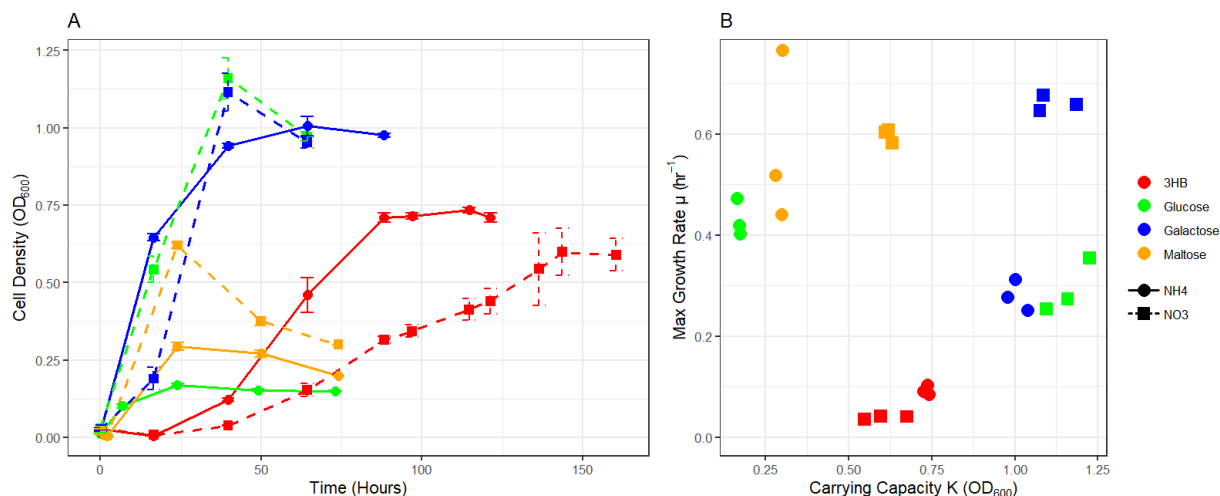


Figure 2. A: OD₆₀₀ Growth Curve Comparison of *A. macleodii* ATCC 27126 on experimental nutrient regimes; B: 2-D comparison of each regime's growth statistics, Carrying Capacity (K) and Max Specific Growth Rate ($\mu_{\max}$).

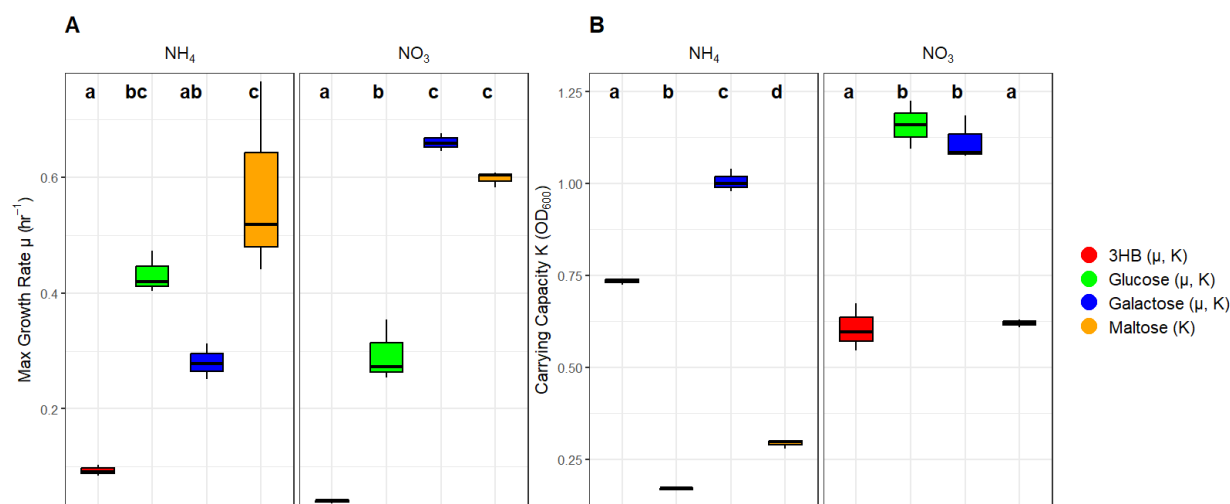


Figure 3. A: Growth Statistic Comparison for $\mu_{\max}$ relative to Nitrogen source; B: Comparison for $\mu_{\max}$ relative to Nitrogen source; values that are significantly different as a result of Carbon source are annotated with a different letter(s); values that are significantly different as a result of Nitrogen Source are annotated with a μ and/or K right of legend.

Increased carbon availability for 3HB (1.5x 3HB in standard experimental cultures) and decreased carbon availability (0.5x Maltose in standard experimental cultures) enhanced the carrying capacity difference induced by nitrogen source (Fig. 4).

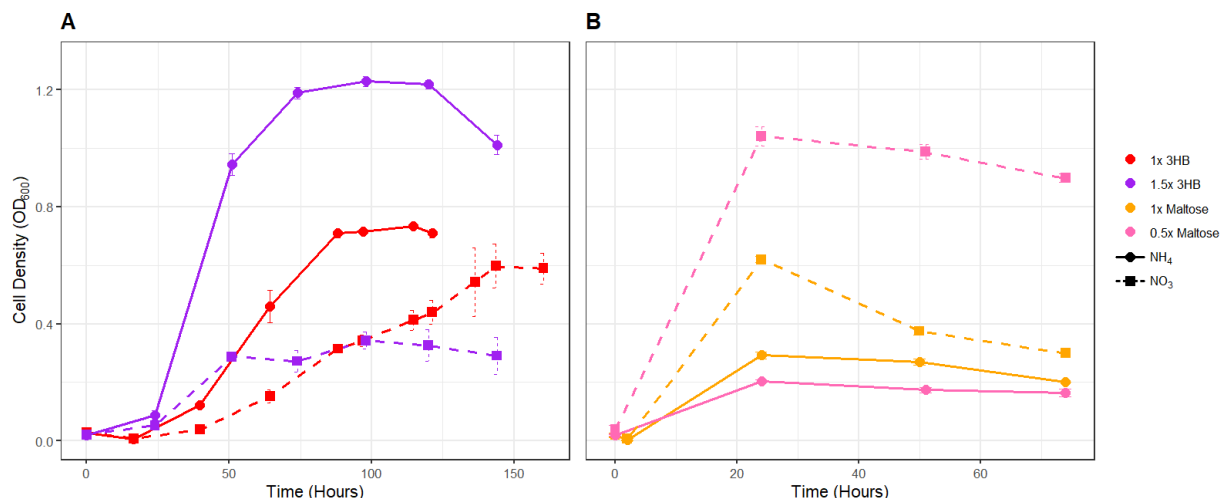


Figure 4. A: Growth Curve Comparison of *A. macleodii* ATCC 27126 on either 1x or 1.5x 3HB concentration and NH₄ or NO₃; B: Growth Curve Comparison on either 1x or 0.5x Maltose and NH₄ or NH₄; Circle data points that follow a solid line are with NH₄ and square data points that follow a dashed line are with NO₃.

Cell Counts

A visual demonstration comparing the carrying capacity cell counts of *A. macleodii* ATCC 27126 on each experimental nutrient regime is displayed in (Fig. 5). The cell count data generally repeats the pattern observed in the OD₆₀₀ K data, with the exceptions of Glu-N being lower than Gal-N and 3HB-N being lower than Mal-N. 3HB-A demonstrated a noticeably large range of values. Shapiro-Wilk Test for Normal Residuals and Leven's Test for Homoscedacity both passed ($p = 0.641$, $p = 0.730$) and the Q-Q plot demonstrated approximate residual normality with subtle deviations at tails; these diagnostic tests indicate that this data set is appropriate for ANOVA analysis. ANOVA analysis demonstrated a significant effect of carbon source and of interactions ($p = 0.0074$, $p = 0.0026$), and a borderline significant effect of nitrogen source ($p = 0.0175$).

The first EMMS pairwise contrast demonstrated a borderline significant negative effect of NO_3 with 3HB ($p = 0.0224$), a borderline significant positive effect of NO_3 with maltose ($p = 0.0445$) and a significant positive effect of NO_3 with glucose ($p = 0.0016$).

The second pairwise contrast demonstrated a borderline significant positive effect of galactose, over glucose with NH_4 ($p = 0.0202$) and a significant positive effect ranking of galactose, to glucose, and to maltose, over 3HB with NO_3 ($p = 0.0009$, $p = 0.0142$, $p = 0.0308$).

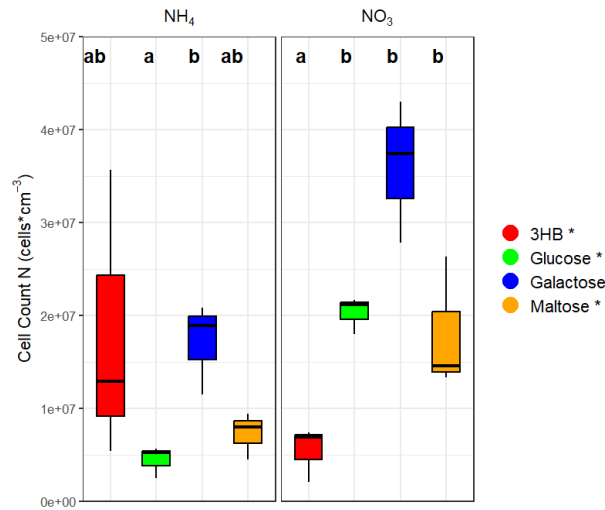


Figure 5. Carrying Capacity Flow Cytometry Cell Count Comparison of Experimental Nutrient Regimes; letter annotations above box and whisker ($n = 3$) indicate significant differences between Carbon sources relative to Nitrogen source; values that are significantly different as a result of Carbon source are annotated with a different letter(s); values that are significantly different as a result of Nitrogen Source are annotated with an asterisk (*) right of legend.

A visual demonstration of the relationship between K and the Cell Counts among each experimental regime is displayed in (Fig. 6). There appears to be a positive correlation.

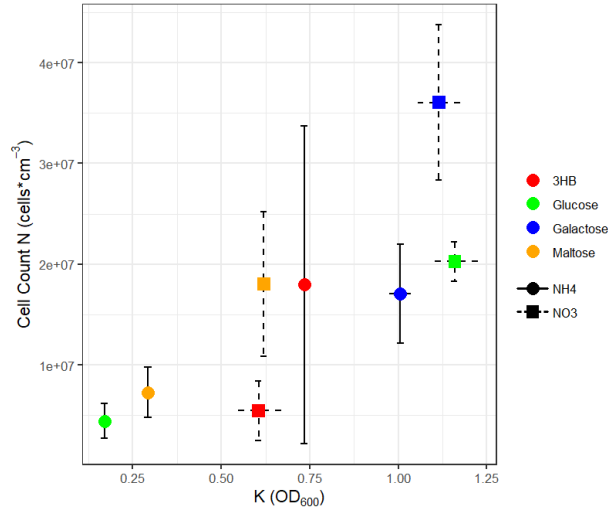


Figure 6. 2-D Comparison of each Experimental Nutrient Regimes' OD₆₀₀ K and Cell Count.

Short Term Oxygen Consumption

A visual demonstration comparing the short term O₂ consumption rate of *A. macleodii* ATCC 27126 on each experimental nutrient regime is displayed in (Fig. 7). O₂ Consumption Rates for Glu-A were unable to be determined due to unmeasurable INT-F precipitation, indicating incredibly low, if any, respiration at the measured time-point. Shapiro-Wilk Test for Normal Residuals and Leven's Test for Homoscedacity both passed ($p = 0.296$, $p = 0.599$) and the Q-Q plot demonstrated approximate residual normality with deviations at both tails; these diagnostic tests indicate that this data set is appropriate for ANOVA analysis. ANOVA analysis demonstrated a significant effect of carbon source and of interactions ($p < 0.0001$, $p = 0.0002$).

The first EMMS pairwise contrast demonstrated a borderline significant negative effect of NO₃ with 3HB ($p = 0.0174$) and a highly significant positive effect of NO₃ with maltose ($p < 0.0001$).

The second pairwise contrast demonstrated a highly significant ranking positive effect of 3HB, to galactose over maltose with NH₄ ($p < 0.0001$) and a highly significant positive effect ranking of glucose, to galactose, and to 3HB, over maltose with NO₃ ($p < 0.0001$).

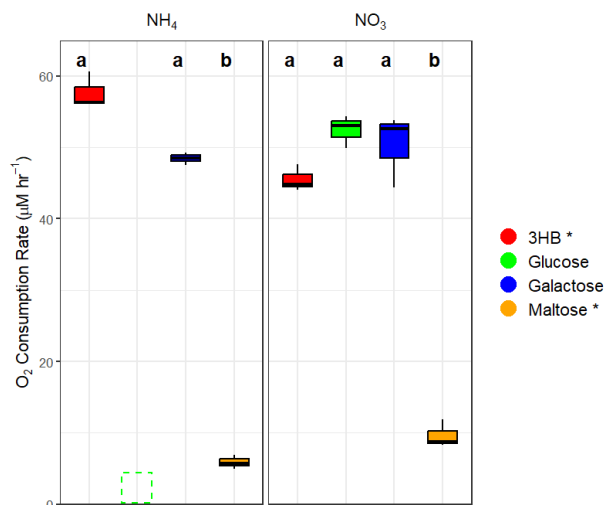


Figure 7. Short-Term O₂ Consumption Rate measured at Carrying Capacity Comparison of Experimental Nutrient Regimes; values that are significantly different as a result of Carbon source are annotated with a different letter(s); values that are significantly different as a result of Nitrogen Source are annotated with an asterisk (*) right of legend; the color-coded box indicates the presumed O₂ Consumption of Glu-A.

Estimated Bacterial Growth Efficiencies

A visual demonstration comparing the BGE_{ES} of *A. macleodii* ATCC 27126 on each experimental nutrient regime is displayed in (Fig. 8). Due to not being able to produce O₂ consumption rates for Glu-A, the BGE_E for this nutrient regime could not be estimated. The BGE_{ES} that could be estimated can be ranked from greatest to least as: Mal-N, Mal-A, Gal-N, Glu-N, Gal-A, 3HB-A, 3HB-N.

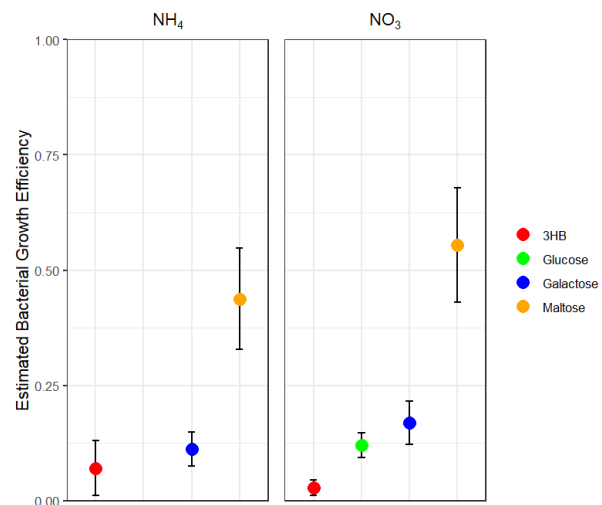


Figure 8. Estimated Bacterial Growth Efficiency Comparison of Experimental Nutrient Regimes; the gap in the NH₄ half is due to no measurable Respiration value from Glu-A.

DISCUSSION

Nitrogen source specificity for carbon utilization

In the absence of any nitrogen source other than that featured in this Thesis, the cells would need to perform de novo synthesis of amino acids in order to build proteins, replicate DNA, and grow on the provided carbon source (Atasoglu et al., 1998). NH_4 is first assimilated by aminating a Citric Acid Cycle (CAC) intermediate, α -Ketoglutarate (AKG), and converting it into the amino acid Glutamate, which can then be converted into Glutamine, or any of the other necessary amino acids to construct proteins and nucleotides (Atasoglu et al., 1998; Goncheva et al., 2022; Nagatani et al., 1971; Reitzer et al., 1979). ATCC 27126 is highly likely able to degrade almost every amino acid, and furthermore, a certain strain of *A. macleodii* has been observed metabolizing amino acids to the degree that it relieves amino acid deficiency in microalgae (Sher et al., 2025; S. Wu et al., 2015). Nitrogen in its oxidized form, NO_3 , cannot readily be incorporated into amino acids in the same way, but must first be reduced to NH_4 in two steps, with nitrite, NO_2 , as an intermediate (Zehr & Ward, 2002). Due to the energy investment required for these pathways, many studies prefer to use NH_4 , but the use of NO_3 has been investigated before (Atasoglu et al., 1998; Diner et al., 2016; Gralka et al., 2023; Hale et al., 2017; Huang et al., 2022; Kimes et al., 2014; Koch et al., 2019; Manck et al., 2022). ATCC 27126 likely utilizes a chimeric Nitrate Assimilation pathway, where NO_3 is transformed into NO_2 via a ferredoxin-utilizing Nitrate Reductase, and NO_2 is transformed into NH_4 via an NAD(P)H-utilizing Nitrite Reductase (Karp et al., 2019; Sher et al., 2025). Despite NH_4 's bioavailability, it is not always preferred as a nitrogen source for optimal growth among different species, or even among different carbon substrates (Albers et al., 1996; Berg et al., 2017; Tays et al., 2018; Zaldívar Carrillo et al., 2018)

The results of this Thesis demonstrate that ATCC 27126 has a preference for NH_4 over NO_3 when grown on 3HB, and the preference is flipped when grown on any of the sugars. The oxidation of 3HB for energy in this bacterium likely follows this pathway: a single molecule of 3HB is converted to Acetoacetate via a NAD^+ -Dependent 3HB Dehydrogenase, which is acetylated into Acetoacetyl-CoA via a NAD^+ -Dependent mechanism of the Fatty Acid Oxidation Complex, which is cleaved into two Acetyl-CoA molecules (Karp et al., 2019; Mierziak et al., 2021; Song et al., 2012). Through the CAC, the two resulting Acetyl-CoA molecules are practically respired into four CO_2 molecules, bringing forth 2 NADPH from the dehydrogenation of Isocitrate into AKG, 4 NADH from the dehydrogenation of AKG and Malate, 2 ATP from the deacetylation of Succinyl-CoA. and 2 FADH_2 from the dehydrogenation of Succinate (Blanco & Blanco, 2022; Karp et al., 2019). Summing those substrate-generated, and those produced via the ATP-Synthase of the ETS, powered by the aforementioned reductants, with the exception of NADPH, a single molecule of 3HB can theoretically result in 18 ATP (Cogliati et al., 2021; Karp et al., 2019). However, 3HB is an organic acid, and in contrast to carbohydrates, is only incorporated into biomass with energy investment. In order to synthesize ribose sugars necessary for DNA, carbohydrates need only be converted to Glucose-6P, achieved early in Glycolysis, and enter the Pentose Phosphate Pathway (PPP), and in order to synthesize carbon skeletons for amino acids and to replenish or expand cellular respiration, carbohydrates need only be oxidized to Phosphoenolpyruvate (PEP), a late intermediate of Glycolysis, and carboxylated into Oxaloacetate (Blanco & Blanco, 2022; Kaleta et al., 2013; Sauer & Eikmanns, 2005). Organic acids are oxidized to Acetyl-CoA and subsequently condensed in the CAC, and although the added carbon is not the same released as CO_2 in the first few steps of the CAC, the only useful intermediates synthesized from net carbon addition are

AKG, an amino acid precursor, and Citrate, which is incorporated into Petrobactin, an iron-chelating siderophore depended on by high organic matter-processing heterotrophic bacteria such as *Alteromonas* (Blanco & Blanco, 2022; Gupta & Gupta, 2021; Manck et al., 2022).

One of the most available pathways for Acetyl-CoA to anaplerotically build up carbon skeletons is the Glyoxylate Shunt, where Isocitrate is split into Succinate and Glyoxylate, forming a new CAC intermediate, and the Glyoxylate is acetylated with another Acetyl-CoA to form Malate; continuing down the CAC from Malate, another NADH is produced, meaning that a single 3HB molecule through the Glyoxylate Shunt results in only 3 NADH, which is approximate to 7.5 ATP (Karp et al., 2019; Kornberg & Krebs, 1957). The Ethylmalonyl-CoA pathway is another known mechanism for marine bacteria to feed anaplerotic reactions, but it tends to be more associated with bacteria that do not possess Isocitrate Lyase, the key Glyoxylate Shunt enzyme, which is expressed by ATCC 27126 (Forchielli et al., 2022; Karp et al., 2019; Manck et al., 2020). Skipping Isocitrate Dehydrogenase in the CAC will negatively impact biosynthetic capabilities, as this enzyme is crucial in cells growing on organic acids for production of the biosynthetic reductant NADPH (Dolan & Welch, 2018). The Glyoxylate Shunt is not an all-or-none switch from the CAC, but rather there is carbon flux partitioning between the two pathways, and therefore the minimum energy produced is likely higher than the aforementioned value and will increase as further anaplerotic reactions become unnecessary and the cell can afford to partition more carbon towards the CAC for energy (Dolan & Welch, 2018). Additionally, Isocitrate Lyase is not as sensitive to substrate as the CAC enzyme, requiring a build-up of Isocitrate to function and effectively making this pathway relatively slower than the standard CAC (Holms, 1996). To carry out the PPP for biosynthetic precursors, new intermediates must undergo the ATP-dependent conversion from Oxaloacetate into PEP (a

cataplerotic reaction that consumes CAC intermediates) and then combine with a second PEP in gluconeogenesis to Glucose-6P, requiring a non-trivial cost of 2 NADH and 4 ATP per Glucose-6P molecule (Karp et al., 2019; Sauer & Eikmanns, 2005). Compared to carbohydrates, growing on an organic acid like 3HB is metabolically expensive for cells, explaining two of our results: the slower growth rate due to the many steps required to build biomass, and the preference for NH_4 over NO_3 (Gralka et al., 2023). Though each NO_3 molecule apparently requires only 3 NADH to be reduced, the need for the cells to synthesize the Nitrate/Nitrite Reductases extends the biosynthetic cost that the culture does not need to face when provided with NH_4 (Buckel & Thauer, 2018; Kaleta et al., 2013; Sher et al., 2025). A simplified summary of the highly oxidizable and readily biosynthetic metabolism of sugars in contrast to 3HB, of nitrogen assimilation, and of the ETS is graphically presented in (Fig. 9).

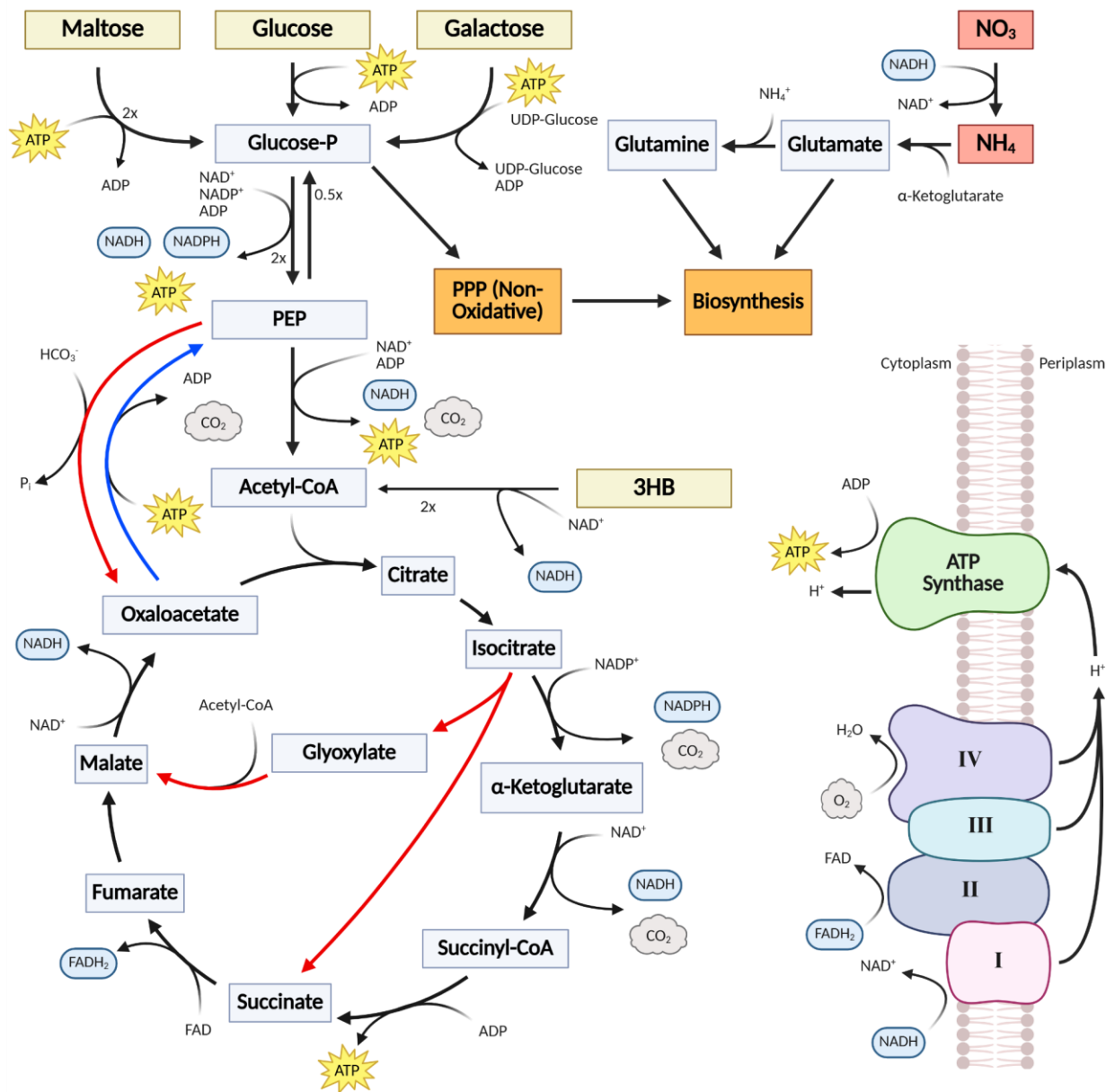


Figure 9. Simplified Summary of Cell Metabolism, represented by the oxidation of 3HB, Glucose, Galactose, and Maltose, the assimilation of Nitrogen, the Citric Acid Cycle with the Glyoxylate Shunt, and the Electron Transport System; Red arrows indicate anaplerotic reactions and blue arrows indicate cataplerotic reactions; note how 3HB has to use the Glyoxylate Shunt and the Oxaloacetate conversion to PEP to enter biosynthesis, in contrast to sugars, where biosynthesis is available almost immediately; many individual steps are not displayed and the cataplerotic biosynthetic entrances for the other CAC intermediates are also not displayed; illustration created with BioRender.

The energy and biosynthetic investment required to reduce NO_3 may also explain the observed trend of paradoxical superior growth on NO_3 with the sugar substrates, through two possible mechanisms: reactive oxygen species (ROS) and/or redox balancing. ROS are leaked by most ETSs, whether involved in photosynthesis or cellular respiration, and can be harmful to cellular growth by disabling ferredoxin complexes, necessary for respiratory enzymes, by damaging DNA strands, and by increasing the signaling for apoptosis, programmed cell death (Fang & Vázquez-Torres, 2019; Foyer, 2018; Giorgio et al., 2007; Imlay, 2015; Latifi et al., 2009). In non-photosynthetic bacteria, most ROS would be produced by the collision of O_2 with non-respiratory flavoproteins, a process known as autoxidation (Imlay, 2013). However, given that there is no evidence that ATCC 27126 expresses any NADH Dehydrogenase II analogues, which are significant ROS-producing sites, and that the relationship between ROS formation and NO_3 reduction is unclear, aside from some forms of nitrate assimilation enzymes having the ability to leak nitrogen radicals, it is unlikely that the production of ROS or other reactive species explains these results (Chen et al., 2022; Messner & Imlay, 1999).

The redox balancing role that NO_3 reduction plays may offer a better explanation. As considered in a recent synthesis article, the redox balance of a metabolic system is a key determinant of reaction favorability, as oxidative reactions, highly represented by carbon catalysis, must be balanced by reductive reactions, often represented by cellular respiration, or else the high NADH/NAD⁺ ratio accumulated will prevent further oxidation, now having become thermodynamically unfavorable (Bouranis & Tfaily, 2024). Depending on respiration to balance redox in high growth conditions is not optimal, as the cell is constrained both by the biosynthetic cost of respiratory enzymes and the limited inner-membrane space for the enzymes, thus cells have developed low-yield overflow metabolisms to increase reductive potential and

cheaply maintain redox balance (Chowdhury et al., 2025; van Hoek & Merks, 2012). These pathways are usually fermentation reactions that generate NAD⁺ or ATP and organic acid byproducts, and though ATCC 27126 likely has the genes for fermentation of Pyruvate to Lactate and Acetyl-CoA to Acetate, the strain's fermentation ability is inconclusive (Karp et al., 2019; Sher et al., 2025). Nonetheless, we hypothesize that the necessity for NO₃ reduction effectively increases the reductive potential, and with the redox balanced, more sugars can be broken down. Thus more biomass can be produced in the presence of NO₃, where this pathway is active, than in the presence of NH₄.

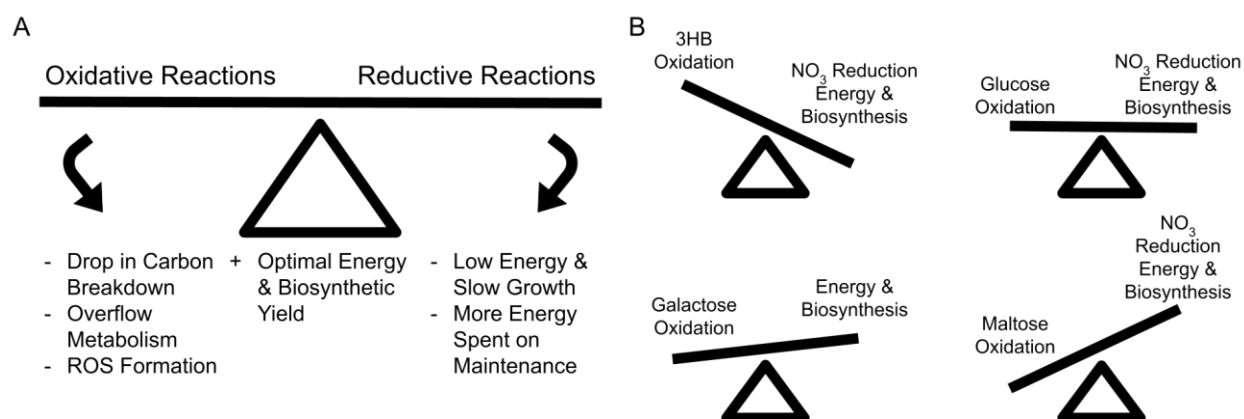


Figure 10. A: Oxidative Reactions are balanced by Reductive Reactions, resulting optimal growth, too much oxidative potential or too much reductive potential results in negative consequences; B: 3HB provides low oxidative potential that can be balanced without NO₃ reduction, Glucose provides a high oxidative potential that is balanced with NO₃ reduction, Galactose provides oxidative potential that can be better balanced with NO₃ reduction, and Maltose provides high oxidative potential that can be offset with NO₃ reduction; adapted from (Bouranis & Tfaily, 2024)

Redox balance is purported to be the explanation for enhanced oxidative metabolism in soil environments with the amendment of electron acceptors, not solely nitrate, but also sulfate, humic acids, and pyrogenic carbon (Bouranis & Tfaily, 2024). Additionally, analogous results of healthier growth on NO₃ over NH₄ were observed in the model angiosperm, *Arabidopsis*

thaliana, with a similar assimilation pathway to that of ATCC 27126 (Dey & Harborne, 1997; Rasmusson et al., 2020). However, if redox balance is posited to be the explanation for this nitrogen-source preference in seawater culture, whether NH_4 or NO_3 is preferred would have to be dependent on the species' natural abilities to manage the redox, with some being more adapted than others; the potential toxic effect of high NH_4 concentrations would also have to be taken into account (Berg et al., 2017; Tays et al., 2018).

Biodegradable Plastic-specific Growth Efficiencies

The cells growing on 3HB were estimated to produce the lowest growth efficiencies among the studied substrates, regardless of nitrogen source supplied. Compared to glucose, organic acids are often incorporated significantly less into biomass per gram of carbon, not able to supply enough energy to transform much of their carbon into cell biomass, instead losing carbon as CO_2 and entering oxidative phosphorylation for energy (Kutscha & Pflügl, 2020; Linton & Stephenson, 1978). Furthermore, the lower growth rates consistently observed with 3HB are known to lower growth efficiency due to extending the time that maintenance costs must be paid (Pirt, 1965). Despite reliance upon the Glyoxylate Shunt which reduces dependence on the ETS and conserves carbon, over time more CO_2 ended up being produced; lower levels of respiration as a result of this pathway may only be observed during lag-phase when the Glyoxylate Shunt is necessary to build biomass. Lower respiration may also be observed under iron-limiting conditions, common in the marine environment, where this pathway has been observed to be upregulated by ATCC 27126, likely to reduce reliance on Fe-requiring ETS and CAC enzymes (Imsande, 1999; J. Li et al., 2024; Manck et al., 2024). Growth on 3HB with 150% concentration of our typical experimental cultures (Fig. 4) yielded OD_{600} carrying capacities similar to Gal-N and Glu-N when provided NH_4 , but growth rates were still slow and

carrying capacities were lower still when provided NO_3 indicating that the 3HB concentration mainly used in this Thesis constrained max possible growth on this substrate. If these results may be extrapolated to the natural water column, it would suggest that organic acid-containing polymers, such as PHB, would be degraded more slowly compared to sugar-containing polymers, yet would contribute less to sinking POC once broken down, and the nitrogen/nitrogen-source availability modulates this utilization preference. It must also be acknowledged that the nitrogen source preference may vary with species (Tays et al., 2018).

Glu-N demonstrated a much higher growth efficiency than 3HB, likely due to its nature as a carbohydrate, but the lack of any measurable respiration from Glu-A at carrying capacity demonstrated that such a specific nutrient regime could not lead to sustained growth. The low growth may be explained by the cells being unable to “pay the redox cost” of effectively transforming glucose into biomass, possibly instead preferring to prioritize survival at low cell densities (Jakowec & Finkel, 2025; Z. Li et al., 2022). *A. macleodii* was observed to grow on glucose when first isolated and has been characterized in many studies as being able to utilize glucose well as a sole carbon substrate, and yet we report that ATCC 27126 cannot utilize glucose very well in the presence of NH_4 (Baumann et al., 1972; Goto et al., 2017; Gralka et al., 2023; Koch et al., 2019). Not all strains of *Alteromonas* manage glucose equally, and a closely related strain was reported to not utilize glucose, potentially due to the absence of glucose transporters (Kimes et al., 2014; Pedler et al., 2014; Pedler Sherwood et al., 2015). Yet our strain when provided with NO_3 was able to rapidly transform glucose into high cell counts, implying that the redox management offered through NO_3 reduction was sufficient for glucose utilization (Goto et al., 2017; Tsuge et al., 2015). Nitrogen source-specific regulation of glucose transporters is unlikely due to detection of nitrogen levels occurring post-assimilation

(Commichau et al., 2006). Glucose as a monomer substrate may be incorporated into biomass and potentially contribute to organic carbon sinkage more than an organic acid substrate like 3HB, even with a reduced nitrogen source, but in the abundance of glucose, an oxidizable nitrogen source would allow for more total incorporation of the carbon into biomass, yet with more CO₂ respired as a cost.

Despite being similar to glucose, galactose was able to consistently yield high cell counts, and though the same nitrogen-source-specific pattern was observed, the differences in growth and respiration are significantly less; the same study (Pedler Sherwood 2015, from now on) that featured low growth and respiration of *Alteromonas* on glucose also reported high values for galactose (Pedler Sherwood et al., 2015). Pedler Sherwood 2015 described their strain as being competent at breaking down glucose, once transported, and at metabolizing other sugars and oligomers, yet was unable to intake specifically glucose, even when supplied with NO₃ (in the form of NH₄NO₃ salt), in contrast to our strain. Galactose is utilized via the Leloir Pathway, which transforms the molecule into glucose through an epimerization that requires another glucose molecule that otherwise could have been broken down (Blanco & Blanco, 2022; Conte et al., 2021; Jiang et al., 2020; Karp et al., 2019). Thus, successful growth on galactose is possibly better explained by the conversion to oxidizable substrate requiring multiple non-oxidative steps that do not produce reductants, presenting the cells with opportunity to manage redox imbalances. Galactose as a monomer substrate leads to high cell counts regardless of nitrogen source, and with O₂ consumption rates comparable to Glu-N it is thus incorporated into biomass as efficiently and may contribute to organic carbon sinkage in a similar way.

In contrast to previous studies that examined *Alteromonas*'s metabolism on disaccharides, this Thesis reports poor growth and respiration with maltose, despite maltose

sharing characteristics with galactose in that they both require a few non-oxidative steps in their conversion to Glucose (Givati et al., 2024; Gralka et al., 2023; Pedler Sherwood et al., 2015). However, maltose, as a simple disaccharide of two glucose molecules, is transformed to glucose much more quickly than galactose, and due to the particular Method design of this Thesis, using equimolar concentrations for all experimental substrates, twice the amount of sugar was effectively available, suggesting a hyper-carbon availability is to blame for the results following the pattern of the glucose regime, yet more severe. Studies that compare substrate utilization often control the carbon content of each substrate and modify the concentrations used accordingly (H. K. Carlson et al., 2020; Givati et al., 2024; Gralka et al., 2023; Pedler Sherwood et al., 2015). Growth on maltose at 50% of the normal experimental concentration yielded OD₆₀₀ carrying capacities similar to growth on glucose at the same carbon concentration, but retained the fast growth rates of maltose, possibly due to intracellular transportation of a single disaccharide yielding more carbon than a single sugar (Arnosti, 2011). However, Pedler Sherwood 2015 did not appear to make such a control for disaccharides, but instead used generally low concentrations of substrate, likely to imitate more realistic concentrations of DOM in the water column (Pedler Sherwood et al., 2015). Regardless, Mal-A led to statistically higher growth than Glu-A, implying that the transformations required to oxidize maltose are more impactful towards redox balance than hyper-carbon availability in some instances. Despite the low respiration measured, Maltose resulted in high growth rates and moderate cell counts, at least in the presence of NO₃, which estimate a high bacterial efficiency and could contribute to organic carbon sinkage at high concentrations, but possibly more at lower concentrations.

The bacterial growth efficiencies estimated in this study cannot be readily compared to literature values, being very low and reflecting the efficiencies of oligotrophic systems despite

deriving from replete conditions (del Giorgio & Cole, 1998). The cell counts incorporated into bacterial growth efficiency were well in the 10^6 - 10^7 cells/mL orders of magnitude observed before in *A. macleodii*, but the per-cell carbon content value of these cells could have been much higher than the value assumed, and thus the BP_{ES} too could have been higher (Givati et al., 2023; Goto et al., 2017; Vrede et al., 2002). However, the respiration rates determined by the INT Reduction method are only estimates of cellular respiration due to the method measuring more than just the activity of respiratory enzymes and requiring a specific oxygen consumption calibration (García-Martín et al., 2019; Maldonado et al., 2012; Seguro et al., 2025). Therefore, it is far more likely that the single respiration timepoint measured at carrying capacity integrated throughout the entire growth time compounded with the INT method's overestimation and led to high BR_{ES} . The assumed substrate-specific Respiratory Quotients used are theoretical and may be less accurate than an experimental quotient. However, experimental quotients do not stray far from their theoretical values and thus wouldn't lead to significant divergences in the BGE_E (Berggren et al., 2012). Natural marine environments are much more heterogeneous with respect to available substrates than the culture nutrient regimes in this Thesis, and such a context can lead to diverging growth regimes from simultaneously assimilating many different substrates, which *Alteromonas* or a comparable diverse microbial consortium is readily capable of (Kovárová-Kovar & Egli, 1998; Pedler et al., 2014). Nonetheless, the results reported indicate clear metabolic differences of *Alteromonas* in response to the carbon and nitrogen sources provided.

The natural concentrations of DIN are often much lower than those used in this Thesis, with NH_4 peaking around 0.25-1.25 μM above 250 m due to rapid scavenging and NO_3 plateauing between 20-40 μM below 1000 m due to the regeneration and nitrification of organic

matter (Casciotti & Buchwald, 2012; GEOTRACES Intermediate Data Product Group, 2025; Zehr & Ward, 2002). This would theoretically lead to different expressions of the nitrogen-source-specific modulation, if not nutrient limitation in some regions (Hale et al., 2017). DIN concentrations closer to those used here may be encountered during instances of significant agricultural runoff, where allochthonous inputs of NO_3 and NH_4 in the euphotic zone stimulate productivity (Edwards et al., 2024; Jin et al., 2023; Zehr & Ward, 2002). Furthermore, concentrations of bioavailable carbon are often much scarcer than those used in this Thesis, which may lessen the need for nitrogen-source-specific redox balance. Regardless, because NH_4 is often at much lower concentrations relative to NO_3 in marine systems, the use of NH_4 in laboratory culture studies with marine bacteria may not be the most reliable due to the apparent importance of redox balance in carbon utilization as indicated in this work.

It must obviously be pointed out that utilization of a monomer, though informative, is not equivalent to degradation of the more complexed polymer (Ren et al., 2025; Yoshida et al., 2016). When studying polymer utilization in a monoculture, the cells first need to invest resources in extracellular hydrolytic enzymes that cleave the polymer into monomers or oligomers that can enter membrane-transport, which may entail much slower growth, but in some instances for naturally occurring saccharides has led to superior growth as a result of multiple possible mechanisms, such as redox balance, or by larger molecules simply yielding more oxidizable substrate (Arnosti, 2011; McBride et al., 2009; Pedler Sherwood et al., 2015; Schink et al., 1992). Should the results for growth on maltose compared to glucose, as a marginally more complexed substrate, be extrapolated to growth on naturally occurring polysaccharides or polysaccharide-containing synthetic polymers, it would imply that a monoculture, specifically of *Alteromonas*, would poorly utilize them. However, because it is far

more likely that this specific finding is an artifact of laboratory conditions that do not properly reflect natural conditions, no informative characterization of polymer degradation in the marine environment can be presented in this respect (Roller & Schmidt, 2015). The degradation of complex substrates in the marine environment is conducted by a much more diverse microbial community, with specific members producing the hydrolyzing extracellular enzymes, and other members benefiting from the bioavailable substrate, despite not contributing enzymes, a group which *A. macleodii* is predicted to be a part of the context of PHB, being able to utilize the monomer, but not able to depolymerize the polymer (Arnosti, 2011; Martínez-Tobón et al., 2018). Present in the water column are also regional and bathymetric differences in hydrolytic activities, with higher rates or a broader range of utilizable substrates in equatorial, coastal, or surface waters; deeper waters however demonstrate higher per-cell activity due to the recalcitrance of deep organic matter (Arnosti, 2011, 2014). Despite the presence of synthetic polymer degraders in the marine environment, the rarity of the more effective degraders typically associated with sediments and composting, combined with frequently low nutrients and low temperatures, PHB remains more recalcitrant than natural polysaccharides and synthetic saccharide-based polymers (Dilkes-Hoffman et al., 2019; Polman et al., 2021).

CONCLUSIONS

If bioplastics are sought as a replacement for conventional plastics, it is significant to characterize how environmental conditions, particularly the available nutrients, impact how they would be degraded and incorporated into natural biomass, along with potential consequences for the carbon cycle. This Thesis explored the metabolism of *A. macleodii* ATCC 27126 on various monomer and oligomer substrates given the more bioavailable NH_4 or the less bioavailable yet more relevant NO_3 through growth curve characterization, cell enumeration, and respiration assays, evidentially supporting the slow growth and low growth efficiency of the organic acid 3HB and discovering a nitrogen source preference dependent on the nature of the carbon substrate provided, with an organic acid inducing a preference for NH_4 , and sugars inducing a preference for NO_3 . These results strengthen the case that organic acid-based biopolymers are more recalcitrant than natural polysaccharides or saccharide-based biopolymers, imply that more PHB-derived carbon would return to the ocean as dissolved CO_2 than be stored in POC, and contribute further to the growing knowledge of *Alteromonas* metabolism, its role in carbon utilization and nitrogen cycling (Sher et al., 2025). The redox-balance hypothesis, once validated with further study, may be applied to the utilization of natural marine organic matter, such as sinking particles with reducing microenvironments, where depleted oxygen restricts further carbon degradation to communities with denitrifiers, or have practical applications on the industrial production of bioproducts, as the oxidation state of the substrates provided to bacterial cultures must be closely examined in order to optimize bioproduction yield (Bianchi et al., 2018; Liu et al., 2018; Roth Rosenberg et al., 2021; Tays et al., 2018).

A weakness of this dataset is that most of the data obtained including: OD_{600} growth rates, cell counts, or INT reduction rates, for any one growth regime are derived from different

culture replicates of ATCC 27126 at different times, which leads to large uncertainties to be propagated when growth efficiency is estimated. With larger culture volumes, all assays may be performed on a single replicate, with aliquots sampled throughout growth to produce robust cell count growth curves and respiration time series, instead of estimates extrapolated from separate OD₆₀₀ growth curves. The INT Reduction Assay incubation may also be further optimized for *Alteromonas*, as artifacts from INT toxicity can appear for prokaryotes in as little as 30 minutes (Seguro et al., 2025). Without proper per-cell carbon content values available for *Alteromonas* in these specific conditions, BGE may be accurately computed through additional CHN Elemental Analysis that can not only inform how each nutrient regime impacts carbon content, but also nitrogen content to inform nitrogen cycling (Fourquez et al., 2014). The hypotheses in this Thesis that attempt to explain the observations may be disproved or further developed with a proteomics or transcriptomics approach that can observe differences in the expression of redox-detecting enzymes, respiratory enzymes, and/or carbon-specific transporters, metabolomics that can provide evidence for or against the overflow metabolism of lactate and pyruvate, and/or an ROS quantification method to check for ROS influence (Bouranis & Tfaily, 2024; Koch et al., 2019; Manck et al., 2020; McBee et al., 2017). Other substrates can also be studied, such as Ethylene Glycol and Terephthalic Acid from Polyethylene Terephthalate (PET) for comparison with another plastic, or longer oligomerized compounds of PHB or polysaccharides to assess how observations change on the size gradient from monomer to polymer.

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