Cosmobiome: Silicon Mobilisation using a diatom-bacteria co-culture for Martian in-situ resource utilisation

Team GEnoM[a*]

[a]IIT Madras, Chennai, India

[*]Corresponding author: igem@smail.iitm.ac.in

Abstract

Long-term space missions, especially human exploration of Mars, face challenges due to the high costs of transporting materials from Earth. In Situ Resource Utilization (ISRU) offers a sustainable alternative by enabling on-site production of essential materials. Our project, Cosmobiome (CO-culture with Silicon Mobilization for BIOmanufacturing using Martian regolith), focuses on a closed-loop biomanufacturing system using a co-culture of silicate-solubilizing bacteria (*Pseudomonas fluorescens*) and diatoms (*Phaeodactylum tricornutum*). This system facilitates silica recycling and dual biomanufacturing for producing chemicals of human interest. We engineered *P. fluorescens* with 4(S)-limonene synthase to produce 4(S)-limonene, a compound with wide industrial applications. Silicate solubilization was demonstrated through quantitative (molybdenum blue) and qualitative assays, while diatom growth was optimized under varying environmental conditions with silicic acid supplementation. Co-culture mutualism was validated by monitoring bacterial CFUs and diatom growth. Metabolic modeling tools such as Flux Balance Analysis (FBA) and parsimonious FBA (pFBA) optimized resource pathways for limonene production. The MICOM framework modeled metabolic interactions between *P. fluorescens* and *P. tricornutum*, showcasing efficient silicon solubilization and uptake. We also developed Astrolabe, a computational tool to identify optimal chassis organisms for ISRU-based synthetic biology. By establishing a mutualistic, closed-loop co-culture system, our approach demonstrates a sustainable solution for ISRU on Mars.

1 Introduction

Background The prospect of long-term space missions, particularly those involving human exploration and colonization of celestial bodies like Mars, faces significant logistical challenges primarily due to the burden of transporting large quantities of resources from Earth. This transportation is both expensive and cumbersome as large amounts of propellant are needed to transport heavier payloads; according to previous research, 7.5 to 13.1 kilograms of propellant are required for each kilogram delivered to the Martian surface, resulting in nearly 10,948.9 metric tons of propellant needed for an average rocket weighing 1,063 metric tons[11]. One potential solution is in-situ resource utilisation which involves using resources from celestial bodies to produce materials on site.This has several benefits: reduces the cost and amount of propellant required by decreasing the size of launch vehicles required for delivery, increases the payload capacity for scientific instruments and equipment since products like propellants are produced in-situ, enables a constant supply of essential resources for long-duration missions reducing the need for extensive resupply missions[**Gerald2005**].

Additionally, biological methods such as synthetic biology, can be used to convert resources from a destination planet into products on-site, using less mass compared to traditional abiotic approaches[15]. Apart from engineering the organisms to get useful products,the organisms themselves can be used in a wide range of applications, for example the silica from the diatoms can be used to make sensors for gases like NO_2 [4] and also can be converted into silicon nanostructures that are used in biosensors, solar cells and other modes of energy storage[1][9].

Project design Previous research has focused on the growth dynamics of diatoms and bacteria in co-cultures, as well as the use of synthetic biology to produce chemicals such as propellants[15][16]. However, few studies have concentrated on creating a co-culture in space to produce valuable molecules. Our project Cosmobiome (CO-culture with Silicon Mobilization for BIOmanufacturing using Martian regolith) aims to develop a closed-loop system through a co-culture of a silicate-solubilizing bacteria and diatom.This closed-loop system facilitates the recycling of silica and enables the extraction of chemicals of human interest through a dual biomanufacturing setup.To prove our proof of concept, we introduced 4(S)-limonene synthase into *P.fluorescens* enabling the organism to en-

zymatically convert geranyl diphosphate to limonene. Further to complement our wet lab experiments we used constraint-based modeling techniques to explore the mechanisms underlying metabolite synthesis in our engineered organisms and also performed community growth analysis to understand the interactions in the co-culture. Another important aspect of ISRU is Chassis selection. For this we created a software-Astrolabe (A Space bioTechnology RecOmmendation aLgorithm for Applications in Biomanufacturing Extraterrestrially) that will provide users with a ranked list of suitable chassis organisms based on environmental resources and host planet conditions.

Hypotheses We first aimed to test our hypothesis that *P. fluorescens* can effectively solubilize silicates found in Martian soil, and that *P. tricorntum* exhibits enhanced growth when exposed to these solubilized silicates. Also, we sought to demonstrate that *P. fluorescens* can be engineered for the biomanufacturing of compounds beneficial to humans, while also establishing that both organisms thrive in a co-culture environment within Martian soil. To predict the effects of introducing synthetic biology pathways on core cellular functions, resource allocation, and energy balance, we employed genome-scale metabolic models.Further, we utilized the MICOM framework to analyze the metabolic interactions [15]occurring within the co-culture of *P. tricorntum* and *P. fluorescens*.

2 Material and Methods

2.1 Wet Lab

Chemicals, Media and Culturing Conditions *Pseudomonas fluorescens* was procured from the Microbial Type Culture Collection (MTCC), Department of Biotechnology, Government of India, and *Phaeodactylum tricornutum* from the Culture Collection of Algae, University of Göttingen. 500g of The Mars Global (MGS-1) High-Fidelity Martian Dust Simulant [12] was obtained from Prof. Sathyan Subbaiah, Department of Mechanical Engineering, IIT Madras, and 100 g of calcium aluminosilicate from Astrra Chemicals, Chennai, India, for further experiments.

Bacteria: Freeze-dried *P. fluorescens* cultures from MTCC were revived, grown at 30°C in a shaking incubator, and stored as glycerol stocks at -80°C. LB plates with calcium aluminosilicate (CaAlSi) were prepared using a slurry of LB, agar, and CaAlSi to evenly distribute the insoluble material.[24][14] An experimental protocol to inoculate P. fluorescens in Martian soil simulant (1:3 mass ratio, 25% moisture) was established after consulting Prof. K. Chandraraj, IIT Madras. The inoculated soil was incubated at 30°C, 250 rpm.

Diatoms: Cultures of *P. tricornutum* were received from the Culture Collection of Algae. Initially, diatoms were inoculated in Mann and Myers medium under static and shaking conditions; no growth occurred in static conditions, and the cultures were discarded. The shaking cultures became turbid but lacked photosynthetic pigments, indicating unhealthy growth. Supplementation with thiamine and cyanocobalamin failed to improve growth, likely due to biotin deficiency and inoculation temperature inconsistencies. BG11 medium was used with microfiltered seawater, with pH maintained between 7 and 8 , to mimic the diatom's natural habitat. [20] Inoculated at 21°C with 410 lux light, the culture reached an OD of 0.14 in five days, indicating healthy growth. Growth conditions were maintained at either 27°C and 160 rpm or 21°C and 150 rpm, with a light dark cycle of 12h:12h and an average light intensity of 1800 lux.

Stressed diatoms were rescued by supplementing them with a carbon source. The culture was grown in f/2 medium supplemented with glycerol, made to a final concentration of 0.1 M.[25] The pH was adjusted to 8.

Minimal media culturing of *P. tricornutum* was studied to enable growth with minimal resources for in situ resource utilization. Diatoms were cultivated in natural seawater supplemented with nitrogen and phosphorus [16] at 27°C, 160 rpm, with a 12h:12h light-dark cycle and 1800 lux light intensity. Growth was monitored by measuring optical density at 750 nm every 24 hours.

Silicon solubisation assay

Qualitative : Two approaches were used to qualitatively confirm the silicate solubilizing activity of *Pseudomonas fluorescens*: The first method involved growing the bacteria on solid growth media supplemented with 0.25% calcium aluminosilicate and subsequently checking for clearance zones around the colonies, which are indicative of solubilization.[24] [14] The second method involved using NBRISSM, a defined differential liquid media for screening silicate solubilizers.[3]

Quantitative : After the bacteria was grown in soil, the solubilized silicates were extracted from soil using calcium chloride.[12]The supernatant was filtered and used for quantification of solubilized silicates using the molybdenum blue method. [10] After 5 minutes following addition of reducing solution, the absorbance was measured at 630 nm using a UV-Vis spectrophotometer. Simultaneously, silica standards of 0.2, 0.4, 0.8, and 1.2

mg L^{-1} were prepared in the same sodium sulfite matrix and had their absorbances measured using the spectrophotometer. [17] After consulting Dr. Deepa Khushalani, we decided that co-relating an increase in diatom growth rate with bacterial presence in soil would be the best way for us to show our proof of concept.

Growth measurements

Bacteria : To inoculate the soil, a 5 ml overnight culture of *P. fluorescens* was centrifuged to collect the cells. The pelleted cells were resuspended in 5 ml of fresh LB media. This cell suspension with fresh growth media was poured into 15 gm of Martian soil simulant (1:3 mass ratio) to achieve a moisture level of 25%. The flask with inoculated soil was placed in a 30℃, 250 rpm shaking incubator. Over a period of 10 days, 1 gm of soil was mixed with 9 ml of sterile water and centrifuged daily. The supernatant was then serially diluted and plated on LB plates. The growth of the bacteria in soil was observed by counting the CFU (colony forming units) every day.

Diatoms : Silicate solubilizing bacteria act by converting the silica present in minerals to silicic acid, which can be utilized by the diatoms in cell wall formation [13].Sodium silicates are one of the few water soluble silicates, and form silicic acid at neutral pH conditions. We test the hypothesis that the diatoms show increased growth in presence of silicic acid in the growth medium by comparing their growth in presence and absence of sodium metasilicate. *P. tricornutum* was cultured separately in minimal medium and minimal medium containing 30 mg/L sodium metasilicate nonahydrate [16] , starting with the same initial OD of 0.05. Diatom cells were then harvested by centrifugation of equal volumes of the stock culture at 8000 rpm for 10 min, and resuspended them in the two media. Growth conditions were maintained at 27°C and 160 rpm. The experiment was carried out in duplicates and the growth was quantified by measuring OD at intervals of 24 hours for 15 days.

Plasmid Assembly and Transformation We chose the constitutive promoter BBa_J23100 and the RBS BBa_J428038 as they have been extensively used and well documented for reliable expression. Our coding sequence was 4(S)-limonene synthase and the terminator was BBa_J428092. The biobricks were transformed into competent *E. coli* DH5α cells. After the plasmids were isolated using miniprep,we used golden gate assembly to assemble the genetic circuit into the destination vector pJUMP24-1A(sfGFP). Successful golden gate assembly was confirmed by selecting colonies that were kanamycin resistant and non-fluorescent. As a second confirmatory test for plasmid assembly, the plasmids were linearized using XbaI and BstI and bands were observed in the agarose gel.

For transformation, *Pseudomonas* was first washed in magensium electroporation buffer and then transformed using the electroporation.[7] Transformation was confirmed by selecting colonies using kanamycin.

Co-culture mutualism *P. tricornutum,* a marine diatom, requires a saline environment for optimal growth, with 30-35% salinity corresponding to 100% seawater. *Pseudomonas fluorescens,* typically grown in low-salt LB media, was tested for tolerance in minimal media with salinities of 30%, 15%, 7.5%, and 5%. Martian soil simulant inoculated with *P. fluorescens* was divided into 3 g samples, each added to four Falcon tubes with 15 ml of minimal media at the different salinities and incubated at 30°C. CFU was measured twice over four days by plating samples on agar and incubating overnight at 30°C. Dense colonies were observed in all salinity conditions during both samplings.

A co-culture of *P. fluorescens* and *P. tricornutum* was designed to facilitate silicate solubilization, carbon cycling, and nutrient exchange. An inoculation ratio of 1:50 (bacteria:diatoms) was chosen based on growth rate differences, confirmed by OD readings at 600 nm (bacteria) and 750 nm (diatoms) and cell counts using a haemocytometer. Cultures were harvested in this ratio, and the co-culture was set up alongside three controls: bacteria alone, diatoms alone, and uninoculated medium. Growth was monitored over time by measuring OD at 600 nm and 750 nm.

2.2 Constraint Based modeling

To complement our wet lab work, we implemented both static and dynamic modeling to represent various aspects of our project.

The overall goal of our modelling was to explore the mechanisms underlying metabolite synthesis using constraint-based approaches.We also analyzed the interactions in a co-culture of Pseudomonas putida and Phaeodactylum tricornutum applying linear constraints to study their combined metabolic behavior. Mathematical modeling in this context allowed us to predict and better understand these biological processes in silico.

Genome Scale analysis We introduced the gene responsible for the synthesis of limonene, limonene synthase[23], into the existing genome-scale metabolic model of the bacteria and conducted various analyses on it. We leverged the CobraPy package to perform the various analysis on the genome scale models

Flux Balance analysis For our project we performed basic flux balance analysis on the model to optimize the

production of limonene to analyze the flow of metabolites across the different pathways.[18] Mathematically FBA consists of

Stoichiometric matrix: S_{ij} and *Flux vector* $\mathbf{v}$

Objective function: A linear function that defines a biological goal mathematically represented as:

$$Z = \mathbf{c}^T \cdot \mathbf{v}$$

Constraints: FBA constists of 2 constraints, the steady state asumption

$$(1)\ S \cdot \mathbf{v} = 0$$

$$(2)\ v_{\min} \leq v \leq v_{\max}$$

Flux Variance analysis: FVA was performed on the model to understand alternate pathways to produce limonene. This also highlighted critical reactions and flexible pathways, providing crucial insights into optimizing the bacteria's metabolism for efficient bioproduct synthesis on Mars[8]

Parsimonious Flux Balance analysis In our project, pFBA was used to refine the metabolic network of the bacteria, allowing us to predict the most resource-efficient pathways for both biomass production and limonene synthesis. An additional constraint to perfrom pFBA was to Minimize $\sum_{i=1}^{n} |v_i|$ where v_i is the flux of the i-th reaction.

Metabolic Optimization and Modeling Analysis This method allowed us to predict the immediate metabolic adjustments following the previously mentioned genetic changes[21]

Regulatory On/Off Mechanism : This method was used to fine tune the bacteria's metabolic network in response to genetic modifications, such as those aimed at enhancing biomass production.

FSEOF We used the FSEOF algorithm [19] to understand the key pathways involved in the production of limonene, by systematically increasing the flux towards acetaminophen production, FSEOF meticulously scanned the entire metabolic network, revealing which reactions needed to be upregulated to achieve our production goals. The top 20 overexpression targets were found

Community modeling We leveraged the **MICOM** (Metagenome-Scale Modeling to Infer Metabolic Interactions) framework [6], to model the intricate metabolic interactions within a coculture system composed of the diatom *Phaeodactylum tricornutum* and the bacterium *Pseudomonas fluorescens*. The first step was to model the silicon solubilization process and incorporate the following series of reactions that help capture the complete process from how it is solubilized in bacteria to how it can be taken up by the diatom as part of its biomass. This also incorporated soluble silica into the biomass equation of the diatom.

$$\text{EX\_silica\_e:}\ \ \text{silica} \rightleftharpoons \text{silica} \tag{1}$$

$$\text{SOL\_silica:}\ \ \text{glcn\_c} + 2.0\,\text{h\_c} + \text{silica} \rightarrow \text{soluble\_silica} \tag{2}$$

$$\text{EX\_soluble\_silica\_e:}\ \ \text{soluble\_silica} \rightleftharpoons \text{soluble\_silica} \tag{3}$$

$$\text{Ex\_soluble\_silca\_uptake :}\ \ \rightleftharpoons \text{soluble\_silica} \tag{4}$$

2.3 Software: Astrolabe

Astrolabe was designed to provide a streamlined approach for selecting chassis organisms based on the environmental resources available and the physiological conditions required for their growth. By leveraging extensive biological and metabolic databases, Astrolabe ranks potential chassis organisms according to their resource utilization efficiency and their adaptability to user-defined environmental conditions. The tool aims to reduce the time and effort involved in manual literature reviews and organism screening, enabling researchers to focus on more targeted experimentation.

2.3.1 The Architecture

The architecture of Astrolabe is designed to facilitate the process of selecting the most appropriate chassis organism based on the user's inputs regarding environmental resources and growth conditions. The pipeline, as in Fig 1 involves several stages, which can be broken down as follows:

User Input and Preprocessing To ensure uniformity across all modules, CHEBI has been used as the standard for chemical species identification. Target temperature and pH conditions are inputs specifying physiological conditions.

Metabolite Search and Matching Astrolabe queries the bioinformatic databases BioCyc, UniProt, KEGG, and BRENDA to identify metabolic reactions and enzyme pathways with the resources of interest.

Scoring Function The scoring function is a central component of Astrolabe, providing a quantitative evaluation of each potential chassis organism's suitability for a given task. It integrates data from the metabolite search with physiological data from MediaDive and BacDive, to assign two key scores to each organism: resourcefulness and survivability.

Resourcefulness Score(RS) This score quantifies the ability of an organism to utilize the given resource. It is determined by the number of pathways, reactions, and enzymes associated with the resource in each organism, based on the metabolite search. The total number of pathways for each organism is then normalized to the maximum number of pathways identified in a single organism across all the databases comparability.

Survivability Score(SS): The survivability score evaluates how well an organism's optimal growth conditions, such as temperature and pH, align with the user's specified conditions by their normalised extent of overlap. This score is derived from data provided by the BacDive and MediaDive databases.

$$h = \sum_{databases} pathway\_hits$$

$$RS = \frac{h}{h_{max}}$$

$$SS = \frac{len(itr \cap otr)}{len(otr)} + \frac{len(ipr \cap opr)}{len(ipr}$$

$$Score = \alpha \times RS + \beta \times SS$$

where itr and otr are the input and organism temperature ranges and ipr and opr are the input and organism pH ranges, respectively.
The output is a ranked list based on the final score, the weighted sum of RS and SS.

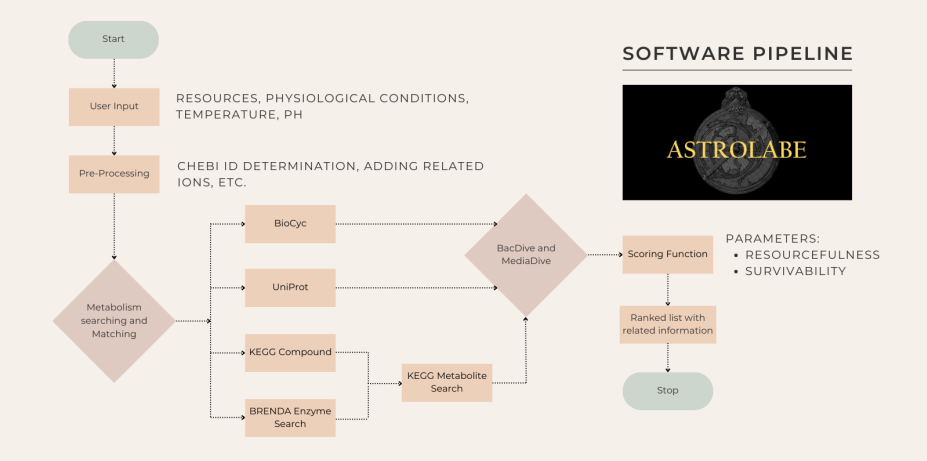


Figure 1: Schematic of Software architecture

3 Results

3.1 Wet Lab

P.fluorescens can solubilize silicates from calcium aluminosilicate in martian soil simulant: At the end of 7 days, the change in colour from purple to yellow in the tube with NBRISSM medium, silicates and bacteria and no change in colour in the control tubes as shown in Figure 2. This suggests that pH changed due to bacteria's silicate solubilization activity.

Growth of *P.fluorescens* in MSS: The bacterial growth dynamics in Martian soil simulant shows a wave-like pattern indicating cycles of growth and death ,as shown in Figure 3 ,which is consistent with the results in [22]

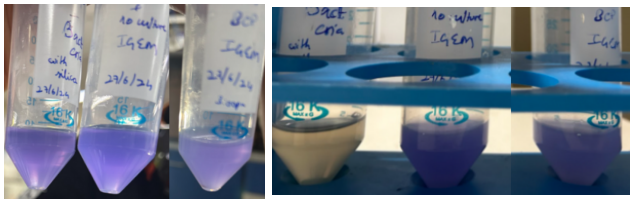
Growth pattern of *P.tricorntum*: The diatoms when grown in minimal medium with only nitrogen and phosphate sources showed a typical growth curve with an exponential phase between days 5 and 8.

Growth enhancement of *P.tricorntum* in the presence of solubilized silica: Presence of silicate in the media leads to higher overall growth of *P.tricorntum* as shown in Figure 5. This suggests that the diatoms can take up solubilized silica from the co-culture environment for cell wall formation.

Plasmid assembly confirmation : The expected fragment sizes after linearization and digestion with XbaI and BstBI were 2 kb and 3.5 kb, which was successfully validated through gel electrophoresis.

Transformation confirmation: Colonies were present on the experimental plate, whereas no colonies were observed on the negative control plate, indicating

successful transformation. All the colonies on the low colony-forming unit (CFU) plate were non-fluorescing while several colonies on the high CFU plate were non-fluorescing confirming successful transformation.



(a) Day 1 (b) Day 7

Figure 2: Qualitative silicon solubilisation assay results-inoculated media with silicates (left), uninoculated media with silicates (middle) and inoculated media without silicates (right)

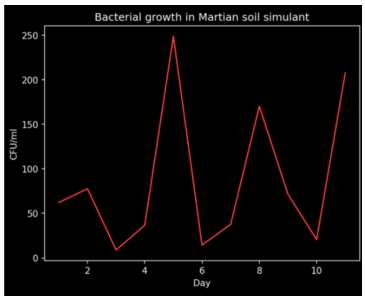


Figure 3: Bacterial Growth in Martian Soil Simulant

Figure 4: *P.tricornutum* in BG-11 made with freshwater (left) with seawater (right). The brown colour is due to the growth of the diatoms.

Salinity optimisation for co-culture: The plates incubated with samples from all four cultures showed dense colony formation. This shows that the salinity of media used in the co-culture setup does not hinder the growth of *P. fluorescens*.

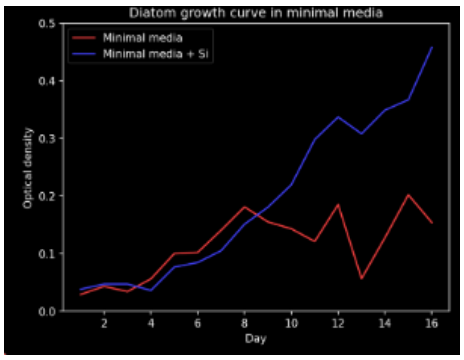
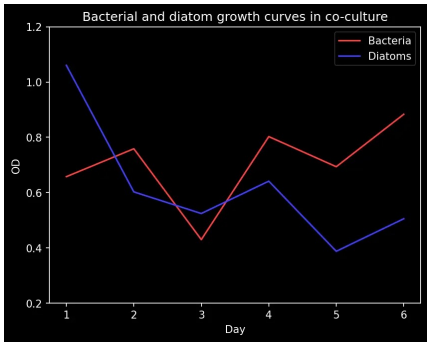
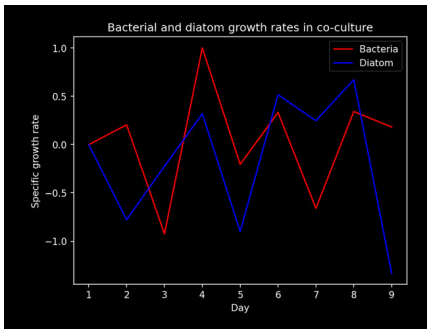


Figure 5: Effect of presence of silicon on diatom's growth



(a)



(b)

Figure 6: (a) OD and specific growth rate (b) measurements of the co-culture

Co-culture mutualism The bacteria and diatoms in the co-culture containing the Martian soil simulant show synchronous growth, with a wave-like pattern corresponding to growth and death cycles of the bacteria in soil, implying a co-dependence of the two species for resources. This is shown in Figure 6. *P. fluorescens* and *P. tricornutum* also show a higher average growth in the co-culture environment as opposed to when grown separately. These results indicate the establishment of a mutualistic relation between *P. fluorescens* and *P. tricornutum* in the co-culture environment

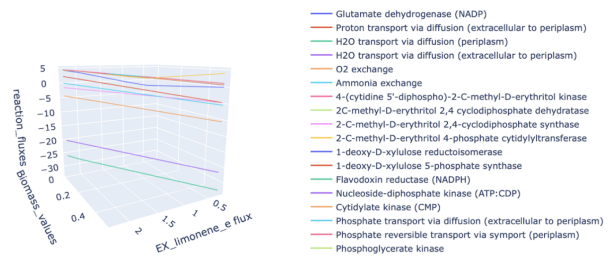
3.2 Constraint based modeling

Genome scale analysis:

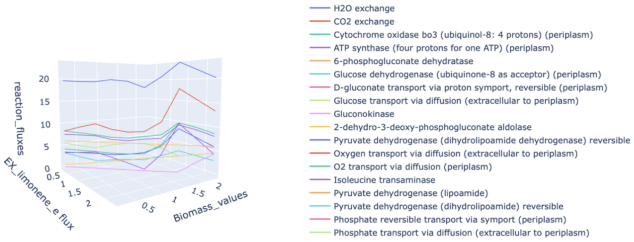
While optimizing limonene production, we observed that the flux for geranyl diphosphate originates from the DMATT(Dimethylallyltranstransferase) reaction and is directed toward limonene synthesis. In the case of biomass production, the same pathway is activated, but the flux is significantly lower, indicating that although geranyl diphosphate plays a role in biomass formation, its impact is minimal, and the flux can remain low while still being essential.

We observed that several common pathways are triggered during the optimization of limonene synthesis. including the reaction ATPS4rpp (ATP synthase reaction) which is crucial for biomass production, while it plays a much smaller role in limonene production, as its flux is significantly lower. Additionally, certain reactions, such as NADH16pp (NADH dehydrogenase reaction) and the diffusion of oxygen into the periplasm, show little to no activation, with minimal flux. Overall, although most of the same pathways are activated, Their influence on the final product's production varies.

FSEOF algorithm: With the help of the FSEOF algorithm we found overexpression targets for limonene synthesis as well as for biomass production as shown in Fig 7



(a) Top 20 FSEOF Targets when biomass is optimized



(b) Top 20 FSEOF Targets when limonene is optimized

Figure 7: (a) FSEOF targets when biomass is optimized. (b) FSEOF targets when limonene is optimized.

The top overexpression reaction targets were identified based on their ability to induce the most significant changes in the flux of the final product. Specifically, the reactions ranked highest in the FSEOF analysis are those that exert the greatest influence on the production of the metabolite of interest.

3.3 Community modeling

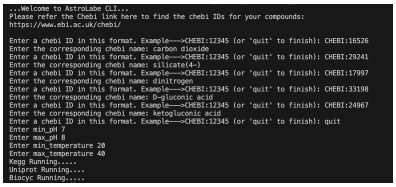
Our hypothesis that increase in the bacteria's growth directly correlates with the diatom's growth was seen in silico when plot was made with growth rate against the tradeoff value We can see as we increase the cooperativity amongst the microbes.

We also showed that The ratio of the biomass flux matches with that of the OD with high accuracy which suggests that the co-culture model is quite accurate.

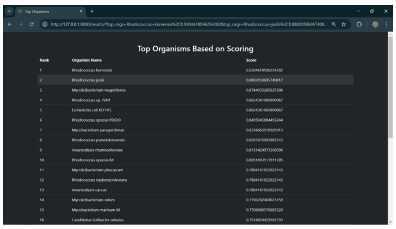
Finally we clearly showed that the growth of the diatom is greater in presence of solubilized silica which is available due to the solubilizing abilities of the bacteria.

3.4 Software: Astrolabe

Astrolabe's output provides a ranked list of organisms based on the combined resourcefulness and survivability scores. The tool calculates these scores by evaluating how well an organism can utilize the specified resource and how compatible its growth conditions are with the user's input. The resulting list includes information about each organism's metabolic pathways, enzymes, and potential applications in ISRU. This ranking system allows researchers to quickly identify the most promising chassis organisms for their experiments, saving valuable time and effort in the early stages of their work.



(a) Astrolabe CLI package

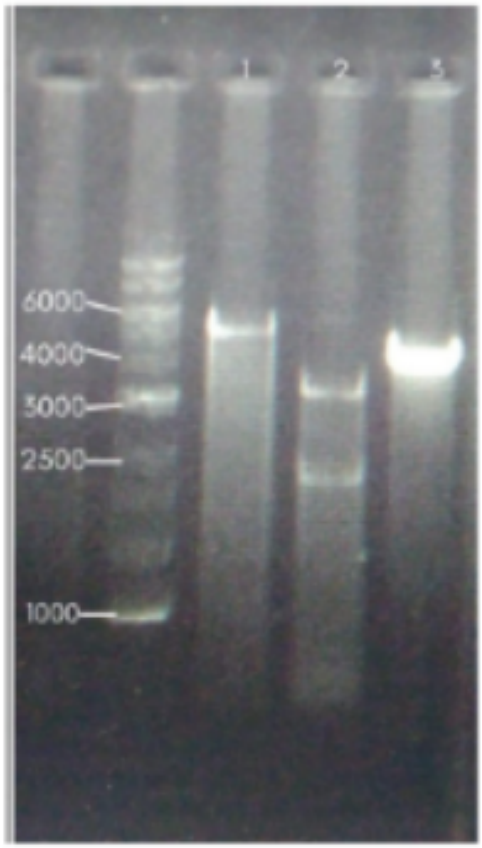


(b) Astrolabe Web tool

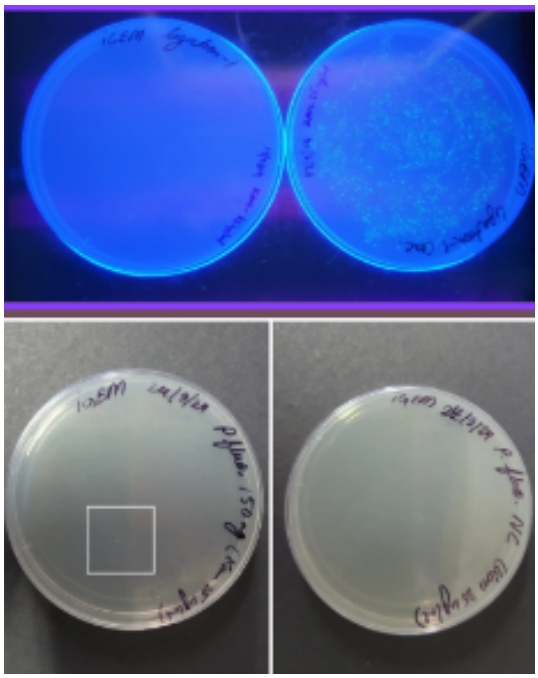
Figure 8: Screenshots of the software tool Astrolabe

We have created two versions of the software package: as a Django web application and a software package for use in a text-based CLI. The web tool is designed to be compatible with most modern browsers, including

Google Chrome, Safari, Firefox, and Opera. By hosting the tool online, researchers can access it from any device with an internet connection, making it more convenient for a wider audience. The CLI package is more suited to automation and modification for specific use cases.



(a) Single digestion of assembled plasmid(1), double digestion of assembled plasmid(2), and single digestion of pJUMP24-1A(sfGFP)



(b) The right plate is the low CFU plate and the left plate is the high CFU plate under UV. A transformed *P.fluoroscens* colony was observed while no colonies were observed on the negative colony plate

Figure 9: Plasmid engineering and Transformation

4 Discussion

In this project, we have established that the *P.tricorntum* and the *P.fluorescens* are mutually dependent on each other as demonstrated by the coordinated and enhanced growth in the co-culture .The co-culture is a closed loop system since *P.fluorescens* converts silicates into soluble silica which is used by *P.tricorntum* to make their cell wall.We hypothesize that the increased bacterial growth may be due to the nutrients released by the diatoms after their death which are taken up by *P.fluorescens* We have also shown the bacteria can be genetically engineered to produce limonene,a compound used in wide range of compounds like pharmaceuticals,food supplements etc. Our model results also helps support the wet lab findings as it gave supporting data to show that the the growth rates of the bacteria and the diatom are correlated with increasing cooperativivty.We also created a strong workflow to help researchers analyze genome scale models when additonal genes are introduced.We have made some assumptions to introduce solubilized silica into the genome scale models of the bacteria and the diatom whose correctness can be a subject to discussion We have also created a software that provides a list of organisms based on how compatible its growth conditions are with the inputs provided by the user and how well it can utilize resources. This can significantly reduce the literature review needed to choose an organism.

5 Future Work

Reactor Setup Our co-culture biomanufacturing system is designed for applications on Mars, requiring a bioreactor capable of withstanding its harsh environ-

ment, including low light, extreme temperatures, and radiation. To address photosynthetic light needs, heliostatic mirrors can concentrate Martian sunlight (43% of Earth's intensity) using a parabolic mirror[5]. Insulating the bioreactor with Martian regolith provides thermal and radiation shielding, while light pipes or periscopes allow light entry[26]. A lightweight reactor made of materials like polyethylene or ETFE, equipped with solar-powered systems for stirring, pH control, and biomass extraction, ensures efficiency[2]. Future modeling under Martian conditions will optimize this scalable design for deployment

Kill-Switch The ReIE-ReIBE toxin-antitoxin system in *Streptococcus pneumoniae* can be repurposed as a kill switch for *P. fluorescens* by replacing its native promoter with a tetracycline-repressible promoter. This promoter includes the gene for the tetracycline repressor protein (TetR) fused with the VP16 activation domain. In the absence of tetracycline, TetR binds to the tetracycline operator, enabling ReIE toxin production and killing the bacteria. In the presence of tetracycline, TetR binds to the antibiotic, inhibiting transcription and preventing bacterial death.

Software Validation The future of Astrolabe includes validating organism selection by comparing its predictions with experimental data on growth rates and metabolic efficiency. Feedback from such studies will refine its database and improve accuracy. Collaborating with database curators and utilizing advanced omics technologies will further enhance its reliability.

6 BioSafety

Our research has been conducted in a Bio-Safetly Level 1 Lab at IIT Madras under supervision from professors and PhD scholars. Both Phaeodactylum tricornutum and Pseudomonas fluorescens are are classified as low-risk and non-pathogenic. For Martian soil simulants, we ensure that materials are sterilized prior to and after use, eliminating any potential cross-contamination. All plasmids and genetic parts used in the project were carefully sourced through the iGEM Registry or a company (Twist Biosciences) following the International Gene Synthesis Consortium (IGSC) guidelines.

Declaration of Conflict of Interest

All authors declare that they have no conflicts of interest.

References

[1] Zhihao Bao et al. "Chemical reduction of three-dimensional silica micro-assemblies into microporous silicon replicas". In: *Nature* 446.7132 (Mar. 2007), pp. 172–175. ISSN: 1476-4687. DOI: 10.1038/nature05570. URL: http://dx.doi.org/10.1038/nature05570.

[2] Philipp Benner et al. "Lab-scale photobioreactor systems: principles, applications, and scalability". In: *Bioprocess and Biosystems Engineering* 45.5 (Mar. 2022), pp. 791–813. ISSN: 1615-7605. DOI: 10.1007/s00449-022-02711-1. URL: http://dx.doi.org/10.1007/s00449-022-02711-1.

[3] Vidisha Bist et al. "Silicon-Solubilizing Media and Its Implication for Characterization of Bacteria to Mitigate Biotic Stress". In: *Frontiers in Plant Science* 11 (Feb. 2020). ISSN: 1664-462X. DOI: 10.3389/fpls.2020.00028. URL: http://dx.doi.org/10.3389/fpls.2020.00028.

[4] Luca Boarino et al. "NO2 monitoring at room temperature by a porous silicon gas sensor". In: *Materials Science and Engineering: B* 69–70 (Jan. 2000), pp. 210–214. ISSN: 0921-5107. DOI: 10.1016/s0921-5107(99)00267-6. URL: http://dx.doi.org/10.1016/S0921-5107(99)00267-6.

[5] Miguel Catela. "Photoresonant plasma induced by solar radiation". In: (2021).

[6] Christian Diener, Sean M. Gibbons, and Osbaldo Resendis-Antonio. "MICOM: Metagenome-Scale Modeling To Infer Metabolic Interactions in the Gut Microbiota". In: *mSystems* 5.1 (Feb. 2020). Ed. by Nicholas Chia. ISSN: 2379-5077. DOI: 10.1128/msystems.00606-19. URL: http://dx.doi.org/10.1128/msystems.00606-19.

[7] Lucy Drury. "Transformation of Bacteria by Electroporation". In: *Basic DNA and RNA Protocols*. Humana Press, pp. 249–256. ISBN: 089603402X. DOI: 10.1385/0-89603-402-x:249. URL: http://dx.doi.org/10.1385/0-89603-402-x:249.

[8] Bruna de Falco et al. "Metabolic flux analysis: a comprehensive review on sample preparation, analytical techniques, data analysis, computational modelling, and main application areas". In: *RSC Advances* 12.39 (2022), pp. 25528–25548. ISSN: 2046-2069. DOI: 10.1039/d2ra03326g. URL: http://dx.doi.org/10.1039/d2ra03326g.

[9] Shubham Gupta et al. “Integration of silicon nanostructures for health and energy applications using MACE: a cost-effective process”. In: *Nanotechnology* 35.42 (Aug. 2024), p. 423001. ISSN: 1361-6528. DOI: 10.1088/1361-6528/ad59ad. URL: http://dx.doi.org/10.1088/1361-6528/ad59ad.

[10] G H Korndörfer et al. “Calibration of soil and plant silicon analysis for rice production”. In: *J. Plant Nutr.* 24.7 (June 2001), pp. 1071–1084.

[11] Shreya Mane. “In-Situ Resource Utilization for Moon and Mars System”. In: *International Journal of All Research Education and Scientific Method* (2022).

[12] *Mars global (MGS-1) high-fidelity martian regolith simulant*. en. https://spaceresourcetech.com/products/mgs-1-mars-global-simulant. Accessed: 2025-1-16.

[13] Véronique Martin-Jézéquel, Mark Hildebrand, and Mark A. Brzezinski. “SILICON METABOLISM IN DIATOMS: IMPLICATIONS FOR GROWTH”. In: *Journal of Phycology* 36.5 (Oct. 2000), pp. 821–840. ISSN: 1529-8817. DOI: 10.1046/j.1529-8817.2000.00019.x. URL: http://dx.doi.org/10.1046/j.1529-8817.2000.00019.x.

[14] J Mary Isabella Sonali et al. “Application of a novel nanocomposite containing micro-nutrient solubilizing bacterial strains and CeO2 nanocomposite as bio-fertilizer”. en. In: *Chemosphere* 286.Pt 3 (Jan. 2022), p. 131800.

[15] Amor A. Menezes et al. “Towards synthetic biological approaches to resource utilization on space missions”. In: *Journal of The Royal Society Interface* 12.102 (Jan. 2015), p. 20140715. ISSN: 1742-5662. DOI: 10.1098/rsif.2014.0715. URL: http://dx.doi.org/10.1098/rsif.2014.0715.

[16] Fiona Moejes et al. “Dynamics of the Bacterial Community Associated with Phaeodactylum tricornutum Cultures”. en. In: *Processes (Basel)* 5.4 (Dec. 2017), p. 77.

[17] C. Narayanaswamy and N. B. Prakash. “Calibration and Categorization of Plant Available Silicon in Rice Soils of South India”. In: *Journal of Plant Nutrition* 32.8 (July 2009), pp. 1237–1254. ISSN: 1532-4087. DOI: 10.1080/01904160903005970. URL: http://dx.doi.org/10.1080/01904160903005970.

[18] Jeffrey D Orth, Ines Thiele, and Bernhard Ø Palsson. “What is flux balance analysis?” In: *Nature Biotechnology* 28.3 (Mar. 2010), pp. 245–248. ISSN: 1546-1696. DOI: 10.1038/nbt.1614. URL: http://dx.doi.org/10.1038/nbt.1614.

[19] Jong Myoung Park et al. “Flux variability scanning based on enforced objective flux for identifying gene amplification targets”. In: *BMC Systems Biology* 6.1 (Aug. 2012). ISSN: 1752-0509. DOI: 10.1186/1752-0509-6-106. URL: http://dx.doi.org/10.1186/1752-0509-6-106.

[20] Giovanni Luca Russo et al. “Bioconversion of cheese whey and food by-products by Phaeodactylum tricornutum into fucoxanthin and n-3 LC-PUFA through a biorefinery approach”. en. In: *Mar. Drugs* 21.3 (Mar. 2023).

[21] Daniel Segrè, Dennis Vitkup, and George M. Church. “Analysis of optimality in natural and perturbed metabolic networks”. In: *Proceedings of the National Academy of Sciences* 99.23 (Nov. 2002), pp. 15112–15117. ISSN: 1091-6490. DOI: 10.1073/pnas.232349399. URL: http://dx.doi.org/10.1073/pnas.232349399.

[22] A. M. Semenov, A. A. Shatalov, and E. V. Semenova. “Microscopic Counting of the Total Number of Bacteria and Metabolically Active Bacteria in Soil Samples: Their Relationship and Oscillative Dynamics of Number”. In: *Uspehi sovremennoj biologii* 144.1 (Aug. 2024), pp. 111–134. ISSN: 0042-1324. DOI: 10.31857/s0042132424010085. URL: http://dx.doi.org/10.31857/S0042132424010085.

[23] Glenn Turner et al. “Limonene Synthase, the Enzyme Responsible for Monoterpene Biosynthesis in Peppermint, Is Localized to Leucoplasts of Oil Gland Secretory Cells1”. In: *Plant Physiology* 120.3 (July 1999), pp. 879–886. ISSN: 0032-0889. DOI: 10.1104/pp.120.3.879. URL: http://dx.doi.org/10.1104/pp.120.3.879.

[24] N Vasanthi, L M Saleena, and S Anthoni Raj. “Silica solubilization potential of certain bacterial species in the presence of different silicate minerals”. In: *Silicon* 10.2 (Mar. 2018), pp. 267–275.

[25] Valeria Villanova et al. “Boosting biomass quantity and quality by improved mixotrophic culture of the diatom Phaeodactylum tricornutum”. en. In: *Front. Plant Sci.* 12 (Apr. 2021), p. 642199.

[26] Cheng Zhou et al. “Properties and Characteristics of Regolith-Based Materials for Extraterrestrial Construction”. In: *Engineering* 37 (June 2024), pp. 159–181. ISSN: 2095-8099. DOI: 10.1016/j.eng.2023.11.019. URL: http://dx.doi.org/10.1016/j.eng.2023.11.019.