COSMOS: COmmunity and Single Microbe Optimisation System

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ABSTRACT

Bioprocessing harnesses the potential of microbial communities and monocultures to convert renewable resources like agricultural by-products and wastewater into valuable products. While monocultures offer simplicity and control, microbial communities provide metabolic diversity, enabling complex substrate breakdown and cooperative biosynthesis. However, balancing efficiency with stability requires a nuanced approach, as each system presents unique advantages and limitations, particularly under different environmental conditions. To systematically explore the potential of these systems, we developed the COmmunity and Single Microbe Optimisation System (COSMOS), a dynamic computational framework that compares microbial monocultures and communities across diverse fermentation conditions. COSMOS identifies optimal microbial systems tailored to specific carbon sources and environments. Our findings reveal that communities outperform monocultures under harsher environmental conditions, demonstrating the value of cooperative interactions. A key result is the identification of the *Shewanella oneidensis* – *Klebsiella pneumoniae* community as the most efficient producer of 1,3-propanediol under anaerobic conditions. Notably, this aligns with experimental findings, with COSMOS accurately predicting the optimal carbon source concentration and inoculum ratio used in the study. Additional findings highlight the value of communities for nutrient-limited processes and emphasise the importance of computational screening to balance productivity with ease of control. The insights gained from this study offer a roadmap toward optimising microbial systems for sustainable bioprocesses and circular bio-economies.

Keywords: metabolic modelling, genome-scale models, metabolic engineering, constraint-based modelling, microbial consortia, communities, monoculture, bioproduction, biosynthesis, bioprocess

1 Introduction

Biomanufacturing supports sustainable development by converting renewable resources, such as agricultural waste or wastewater, into valuable products like biofuels, pharmaceuticals, and bioplastics (Wang et al. 2023). Both microbial monocultures and communities are used in these processes, each with distinct strengths and challenges. Monocultures are easier to control, manipulate, and engineer for specific product yields, making them ideal for simple, well-characterised bioprocesses. However, their productivity can reach a plateau, even after genetic optimisation (Pachapur et al. 2015).

Microbial communities, on the other hand, leverage complementary metabolic capabilities and interspecies cooperation (Ibrahim, Raajaraam, and Raman 2021). In these systems, organisms break down complex substrates—such as lignin or cellulose—into simpler compounds, facilitating resource-sharing (Peng, Gilmore, and O'Malley 2016; Raajaraam and Raman 2024). Communities are also less prone to feedback inhibition because one species may utilise by-products of another, leading to enhanced growth and stability (Ghosh, Chowdhury, and Bhattacharya 2016). Furthermore, the 'division of labour' across species helps distribute the metabolic burden, improving production efficiency for complex products (Rafieenia, Atkinson, and Ledesma-Amaro 2022). However, communities are inherently harder to manage and require careful optimisation to maintain stability and maximise productivity.

Given these trade-offs, the choice between monocultures and communities is dependent on the nature of the bioprocess. The cooperative behaviour of communities provides resilience and efficiency (Ziganshin et al. 2013), whereas monocultures are well-studied and can be easier to manipulate. Designing effective bioprocesses involves identifying the most suitable microbial system for specific substrates or desired products. The decision isn't always as straightforward as selecting communities for lignocellulosic biomass conversion and monocultures for simpler fermentations. Selecting the optimal microbial system for other processes can be challenging, as it would require testing numerous combinations of organisms and environmental conditions—an approach that is both labour-intensive and impractical. Therefore, computational algorithms become essential for managing this complexity and streamlining the selection process. There are very few algorithms that can help us optimize microbial systems for a particular environment.

For example, FLYCOP (García-Jiménez, García, and Nogales 2018) optimises a specific microbial co-culture for synthesizing the desired product. Other studies, like (Wilken et al. 2018), use constraint-based modelling to find suitable bacterial partners for anaerobic fungi in co-cultures. However, these tools are limited in scope—they cannot comprehensively analyse multiple communities or compare them with monocultures to determine the most effective system. In this work, we present COSMOS, an algorithm that overcomes these limitations and

identifies the best microbial system by building pairwise communities from a set of individual organisms. Both the monocultures and communities are analysed in the *same* environment, and instead of comparing the productivity of the community with the average of that of the monocultures, we compare the highest productivity of either of the monocultures. This helps us identify the *best* system instead of merely evaluating if the community is better than the sum of its parts (Zaramela et al. 2021). While looking at an average makes sense while studying growth and interaction, it is better to look at the end productivity/yield achieved to choose the best system.

COSMOS analyses both communities and monocultures under identical environmental conditions using dynamic Flux Balance Analysis (dFBA) (Henson and Hanly 2014; Mahadevan, Edwards, and Francis J Doyle 2002). While traditional steady-state modelling is simpler to use, it assumes equal growth rates and is better suited to natural communities (Chan, Simons, and Maranas 2017). dFBA accounts for fluctuating growth in synthetic consortia and can give promising results even with the lack of organism-specific parameters, as shown in the following sections (Gomez, Höffner, and Barton 2014). By incorporating Flux Variability Analysis (FVA), COSMOS reduces redundancy in metabolic predictions, offering more accurate insights into productivity (Gudmundsson and Thiele 2010).

COSMOS compares the performance of each community against the highest-performing monoculture, rather than relying on the average productivity of the monocultures, ensuring that the best microbial system is chosen. It also enables users to make informed decisions not only based on productivity but also on additional factors, such as the abundance distribution of the community or any biological insights that might favour one community over another.

In summary, this work contributes to the development of sustainable biomanufacturing by identifying when communities or monocultures should be used and how bioresources can be efficiently employed. It marks a step forward in creating economically viable, eco-friendly processes, aligning with the global effort to promote sustainable industrial practices. This framework aligns with the growing need for optimised bio-based processes that reduce waste and reliance on fossil resources, promoting a circular economy.

2 Methods

2.1 FBA

The metabolic network of the organism is represented as a stoichiometric matrix S of size $m \times n$ where m is the number of metabolites and n is the number of reactions. The entries in each column represent the stoichiometric coefficients of the metabolites that constitute the reaction. The linear programming (LP) problem is denoted by

$$max_v \quad c^T v$$

$$\text{subject to} \quad S.v = 0$$

$$\text{while} \quad v_l \leq v \leq v_u$$

where c is a vector of weights denoting the contribution of each reaction to the objective function, $v \epsilon R^n$ is the vector of metabolic fluxes, v_l and v_u are the lower and upper bounds, respectively.

2.2 Dynamic FBA

Dynamic flux balance analysis is performed using the static optimisation approach where the entire batch time is divided into intervals, and the LP is solved at each time interval as a standard FBA problem. While we assume that the cell maintains an intracellular steady state at any given time point, all variables—both intracellular and extracellular—are subject to time variations. The growth rate (μ) and intracellular fluxes (v), including product secretion rates (v_p) at any given time point are determined by solving a standard Flux Balance Analysis (FBA) problem at the biomass concentration X. Instead of using fixed substrate uptake rates as in the case of classic FBA, we utilise extracellular concentrations of substrates (S) and products (P) to calculate dynamic substrate uptake rates (v_s) based on specific kinetic parameters, as shown in Figure 1. These rates reflect the maximum uptake capabilities at each time point and are applied as constraints in the calculations.

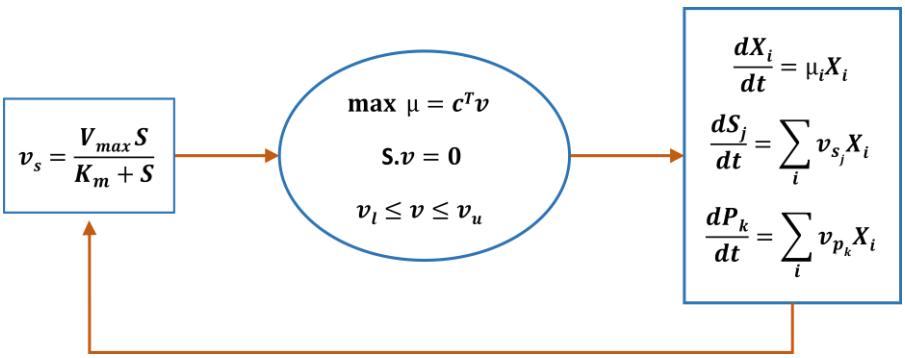

Fig 1. Dynamic flux balance analysis model for a microbial community. μ_i is the growth rate of species i, X_i is the biomass of species i, v_{s_j} is the substrate flux of metabolite j and v_{p_k} is the product flux of product k. The substrate flux is determined by the substrate concentration S and kinetic parameters V_{max} and K_m.

2.2.1 Calculation of substrate uptake rate

The substrate uptake limit is limited by two factors – the amount of nutrient available in the medium for each species i and the transport kinetics for the substrate. We have used a similar approach as (Chiu, Levy, and Borenstein 2014; Varma and Palsson 1994; Wagner 2022) to calculate the substrate uptake rate. The nutrient concentration in the medium is denoted by $S_j(t)$,

where the maximum amount of nutrient j that species i can import per gram biomass per hour is $S_{j,conc}$

$$S_{j,conc} = \frac{S_j(t)}{X_i(t) * \Delta t}$$

The second limitation is transport, where the cell's transport mechanism may not be able to import all the available nutrients. Nutrient transport often follows Michaelis–Menten kinetics (Button 1991; Fiksen, Follows, and Aksnes 2013), and we therefore use the kinetic parameters V_{max} and K_m to calculate the transport limit $S_{j,trans}$

$$S_{j,trans} = \frac{V_{max} * S_j(t)}{K_m + S_j(t)}$$

The substrate uptake rate v_s is the minimum of these two limits and is given by

$$v_s = min\left[\frac{S_j(t)}{(X_i(t) * \Delta t)}, \frac{V_{max} * S_j(t)}{K_m + S_j(t)}\right]$$

This substrate uptake rate is used as an additional constraint for the parsimonious flux balance analysis to compute the growth rate μ_i of each species i and the medium concentration for each substrate is updated.

2.2.2 Flux Variability Analysis

To evaluate the production of each product under study we performed a Flux Variability Analysis (FVA) at each time step. The maximum flux through the exchange reaction for k products v_{p_k} is evaluated as follows

$$max_v \quad v_{p_k}$$

$$\text{subject to} \quad S.v = 0$$

$$\text{while} \quad v_l \leq v \leq v_u$$

The product concentration $P_{k,i}$ for product k in species i is given by

$$P_{k,i} = v_{p_k} * X_i(t) * \Delta t$$

2.3 Comparative analysis

High-quality GSMMs of more than 30 organisms were obtained from the BiGG models database (King et al. 2016) and the BioModels database (Malik-Sheriff et al. 2020). These models were filtered based on pathogenicity, model quality and annotation compatibility (Lieven et al. 2020). We chose ten organisms for our final analysis, and though some of these are pathogenic,

bioproduction has been successfully demonstrated in all of them. These models were used to form pairwise communities with a universal compartment shared by the organisms, representing the external medium. The uptake kinetic parameters V_{max} and K_m were assumed to be constant (V_{max} = 20 mmol/gDW/hr and K_m = 0.05 mmol) for all metabolites as the parameters for all the organisms were not readily available. This value of V_{max} and K_m lies within the broad range reported in the BRENDA database (Scheer et al. 2011) and has been utilised in several previous studies (Chiu, Levy, and Borenstein 2014; Roman and Wagner 2018). The pairwise community and the monoculture growth rates were simulated in the same external medium, and the product concentrations in both cases were compared to find the best system under each scenario and target product, as shown in Figure 2. The final biomass, substrate, and product concentrations are averaged over the last five feasible solutions to ensure robustness. A minimal abundance of 0.1 and at least a 10% increase in biomass is set as a requirement for the community to be considered viable.

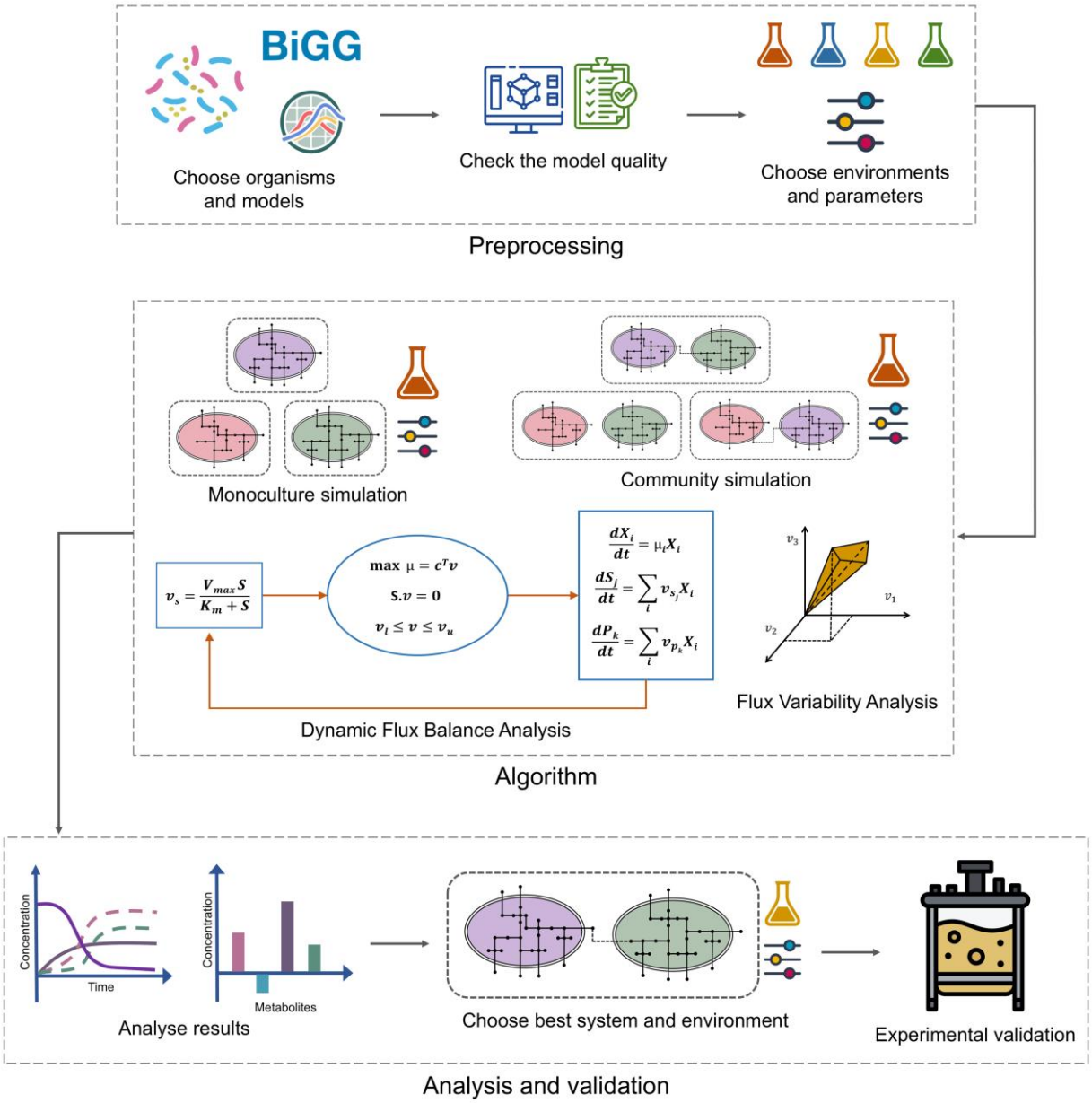

Fig 2. Workflow of COSMOS. The preprocessing step involves selecting target organisms, ensuring model quality, and setting parameters like V_{max}, K_m, initial biomass, and process time. The algorithm uses these inputs to form pairwise communities and applies dFBA and FVA to simulate growth and productivity across mono- and co-cultures in the same environment. The resulting growth, abundance, and productivity metrics help identify the optimal microbial system for the given conditions, which can then be experimentally validated for bioprocess implementation.

3 Results

3.1 Thriving in Scarcity: Communities Excel in Nutrient-Poor Conditions

The medium composition is one of the strongest factors that can affect a bioprocess. We come across both nutrient-dense or 'rich' feedstock like animal manure and sludge, which have more nitrogen content and trace minerals, and 'less-dense' feedstock like wheat straw and corn stover (Mohd Johari et al. 2020), which may lack essential micronutrients. The biosynthetic capability of the organisms can also be affected as a result of this medium composition (Williams et al. 2016). So, we need to either choose the target product based on the feedstock availability or source the best-suited feedstock for our product of interest. Using COSMOS, we analyse the effect of medium composition on the biosynthetic capability of a diverse set of organisms.

To investigate the impact of medium composition, we designed two types of media: a 'minimal medium' and a 'rich medium.' The 'rich medium' contains over 60 metabolites commonly found in growth media (Pacheco, Moel, and Segrè 2019). In contrast, the minimal medium consists of around 40 essential metabolites required for the growth of all the individual organisms under study. A complete list of organisms and media components is provided in Supplementary File 1. The initial concentration of each component was set to 10 mmol/L in both media. The organisms in each pairwise community started with an initial biomass of 0.01 g/L to ensure adequate growth, and every simulation was run for 12 hours, during which the carbon source was fully consumed, and the organisms reached the stationary phase.

We calculated the change in productivity between the two monocultures and their corresponding two-member community. It should be noted that the maximum productivity of the two monocultures was compared against the productivity of the community. This change was classified as an 'increase' if the community demonstrated at least a 10% improvement in productivity compared to the highest-performing monoculture and as a 'decrease' if productivity declined by more than 10% relative to the best monoculture. Any other outcome was categorised as 'no change.' The percentage of total products showing either an increase or decrease in productivity across all environments is presented in Figure 3.

In a community with organisms A and B where

$$Mono_A + Mono_B = Comm_{AB}$$

the change in productivity is given as

$$\Delta Productivity = Productivity(Comm_{AB}) - \max\left(Productivity(Mono_A), Productivity(Mono_B)\right)$$

When comparing the two media types, we found that communities perform significantly better under nutrient-limited (minimal) conditions. This can be attributed to the cooperative interactions within communities, which enhance survival compared to isolated monocultures. A similar observation was documented by (Pacheco, Moel, and Segrè 2019), where microbial communities exhibited more 'costless' interactions (have no impact on growth) when grown in minimal media. Conversely, in nutrient-rich environments, individual organisms tend to perform better on their own, benefiting from abundant resources.

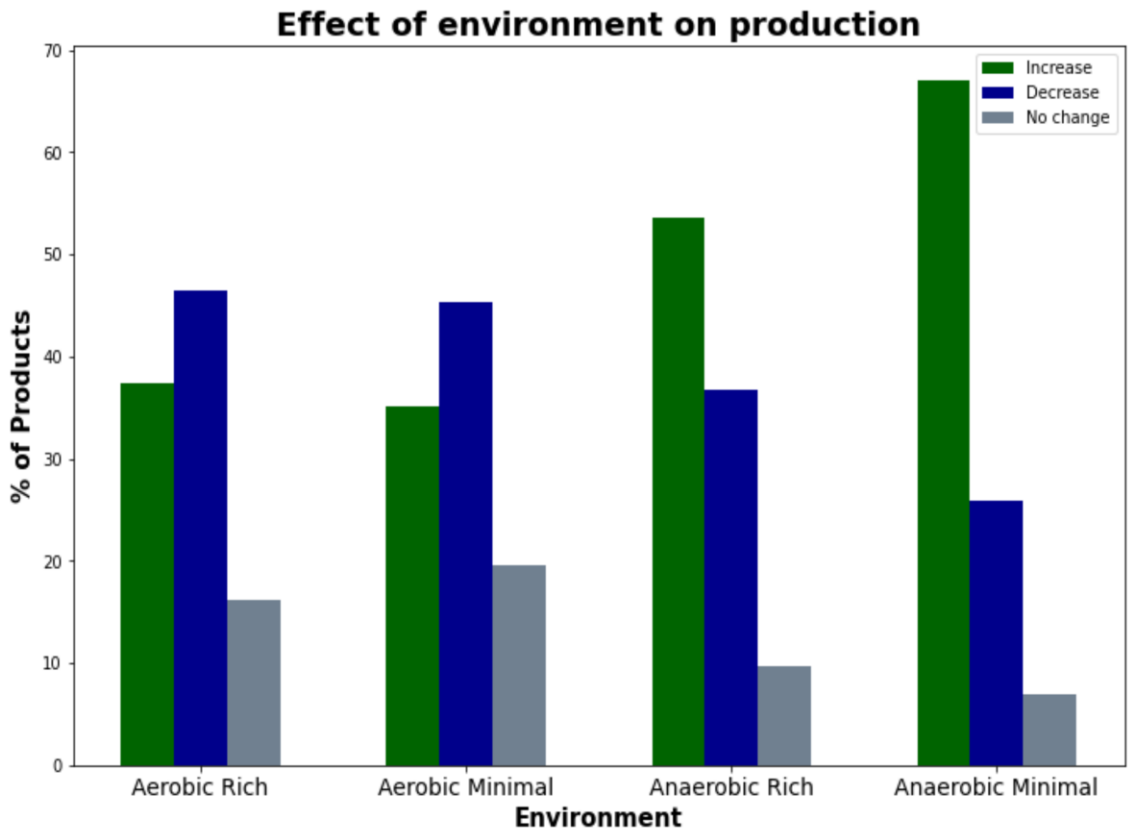

Fig 3. Effect of medium composition and oxygen availability on the productivity of communities vs monocultures. The fraction of products showing increased or decreased productivity when switching from monocultures to communities is illustrated across different environments. An 'Increase' indicates improved productivity in the community, while a 'Decrease' reflects a decline compared to monocultures. 'No change' signifies that the switch results in no significant difference in productivity.

We also examined the maximum product concentrations for four key products from different classes across various environments: fumarate (organic acid), ethanol (alcohol), spermidine (polyamine), and glutathione (tripeptide), as shown in Figure 4. Although maximum product concentration was higher in the nutrient-rich medium, communities consistently showed better biosynthetic ability in nutrient-limited conditions, aligning with our earlier observations.

These findings suggest that, for bioprocesses requiring nutrient-limited media, communities offer a promising alternative to monocultures. However, we can also find some outliers where communities perform very well in nutrient-rich environments and monocultures perform well in nutrient-poor environments. For example, in the aerobic-rich medium, the sorbitol productivity of the *S. cerevisae – B. subtilis* and the *S. cerevisae – L. lactis* communities are the highest and have 59% and 48% improvement, respectively, when compared to monocultures. Likewise, in the anaerobic-minimal medium, *Bacillus* subtilis outperforms all the other microbial communities in the production of L-lactate, with the productivity of B. subtilis being 0.29 mmol/L/hr. It is to be noted that the second-best alternative is *E. coli – P. aeruginosa* community, with a productivity of 0.12 mmol/L/hr of L-lactate, which is less than half of the monoculture. In spite of the general inferences, there can be exceptions which can help us build better bioprocesses. Therefore, a systematic screening of both communities and monocultures becomes necessary. In the following sections, we explore the impact of additional factors, such as oxygen availability and carbon sources, on productivity and community dynamics.

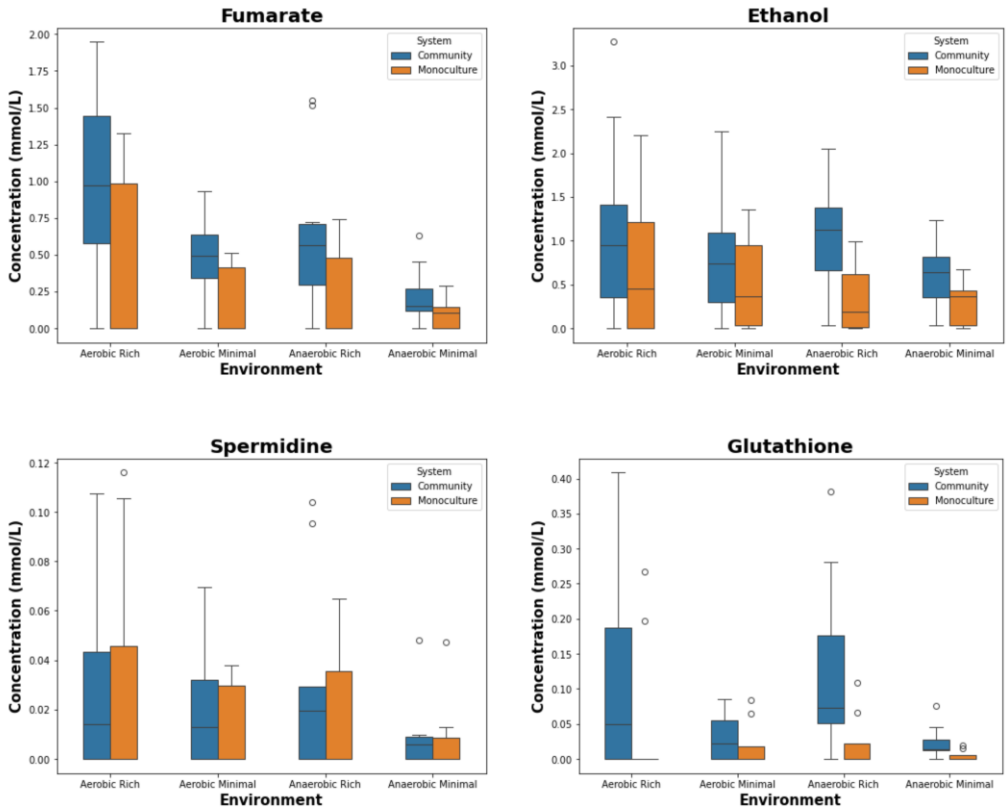

Fig 4. Maximum achievable concentrations of key chemicals across varying environmental conditions. The productivity of both communities and monocultures for specific products across all four environments is represented.

3.2 Anaerobic Advantage: Communities Outperform Monocultures in Anaerobic Conditions

Another critical factor that can affect a bioprocess is the availability of oxygen. To study this, the communities were grown under anaerobic conditions and aerobic conditions with an oxygen concentration of 10 mmol/L for the aerobic environment. The obligate anaerobes and aerobes were excluded from the simulations, respectively (Supplementary File 1). We compared the productivity of the community and monocultures, as shown in the previous analysis.

As shown in Figure 4, the presence of oxygen significantly impacts the biosynthetic capability of both communities and monocultures. While aerobic conditions generally achieve higher product concentrations due to improved growth, communities outperform monocultures under anaerobic conditions, as shown in Figure 3. This could be because aerobic environments promote individual growth, resulting in higher productivity, whereas growth is limited in anaerobic conditions, leading to lower overall productivity.

As observed in the previous analysis, communities perform better in harsher environments like anaerobic conditions, showing improved productivity compared to monocultures. To test this hypothesis, we analysed five communities that grow across all four environments, as shown in Table 2. We found that, under anaerobic and minimal conditions, the abundance of the organisms within a community becomes less skewed. Furthermore, interactions within these communities become more positive, with mutualism and commensalism occurring more in harsher environments. This observation suggests that anaerobic and minimal environments foster greater cooperation among community members.

268 **Table 1. Optimal microbial for four products in the aerobic-rich environment**

#	Fumarate			Ethanol			Spermidine			Glutathione		
	Microbial system	**Productivity (mmol/L/hr)**	**Abundance**	**Microbial system**	**Productivity (mmol/L/hr)**	**Abundance**	**Microbial system**	**Productivity (mmol/L/hr)**	**Abundance**	**Microbial system**	**Productivity (mmol/L/hr)**	**Abundance**
1	*P. aeruginosa - K. pneumoniae*	1.28	0.85,0.15	*S. oneidensis - K. pneumoniae*	2.04	0.19,0.81	*E. coli*	0.07	1	*S. oneidensis - K. pneumoniae*	0.26	0.19,0.81
2	*E. coli - K. pneumoniae*	1.22	0.60,0.40	*S. cerevisiae - K. pneumoniae*	1.42	0.13,0.87	*B. subtilis*	0.05	1	*E. coli - K. pneumoniae*	0.25	0.60,0.40
3	*S. oneidensis - K. pneumoniae*	1.10	0.19,0.81	*K. pneumoniae*	1.29	1	*S. cerevisiae - L. lactis*	0.03	0.84,0.16	*E. coli - P. putida*	0.19	0.72,0.28
4	*E. coli - P. putida*	1.07	0.72,0.28	*E. coli*	1.10	1	*S. cerevisiae*	0.02	1	*E. coli*	0.17	1
5	*B. subtilis - K. pneumoniae*	0.88	0.15,0.85	*P. aeruginosa - S. oneidensis*	0.63	0.89,0.11				*B. subtilis - K. pneumoniae*	0.13	0.15,0.85

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Table 2. Comparison of Microbial Communities Exhibiting Growth Across All Environments

	Aerobic-rich				**Aerobic-minimal**				**Anaerobic-rich**				**Anaerobic-minimal**			
Community	Biomass	Total Biomass	Abundance	Interaction	Biomass	Total Biomass	Abundance	Interaction	Biomass	Total Biomass	Abundance	Interaction	Biomass	Total Biomass	Abundance	Interaction
E. coli - L. lactis	0.76, 0.17	0.93	0.82, 0.18	Commensalism	0.57, 0.13	0.7	0.81, 0.19	Parasitism	0.35, 0.23	0.58	0.60, 0.40	Mutualism	0.19, 0.24	0.43	0.44, 0.56	Mutualism
L. lactis - S. oneidensis	0.13, 0.35	0.48	0.26, 0.74	Parasitism	0.13, 0.36	0.49	0.26, 0.74	Parasitism	0.14, 0.22	0.36	0.38, 0.62	Parasitism	0.13, 0.22	0.35	0.38, 0.62	Parasitism
L. lactis - K. pneumoniae	0.12, 0.80	0.92	0.13, 0.87	Parasitism	0.12, 0.35	0.47	0.15, 0.85	Parasitism	0.13, 0.48	0.61	0.21, 0.79	Parasitism	0.14, 0.29	0.43	0.32, 0.68	Parasitism
B. subtilis - K. pneumoniae	0.14, 0.80	0.94	0.15, 0.85	Parasitism	0.14, 0.66	0.8	0.18, 0.82	Parasitism	0.17, 0.46	0.63	0.27, 0.73	Parasitism	0.17, 0.29	0.46	0.37, 0.63	Commensalism
S. oneidensis - K. pneumoniae	0.19, 0.78	0.97	0.19, 0.80	Commensalism	0.15, 0.64	0.79	0.19, 0.81	Parasitism	0.23, 0.48	0.71	0.32, 0.68	Mutualism	0.25, 0.27	0.52	0.47, 0.53	Mutualism

3.3 Synergy in Action: Positive Interactions Drive Higher Productivity

As we are dealing with pairwise communities, another interesting factor that we can analyse is the effect of interaction. To find the interaction type of a community, we compare the growth of the organism as a monoculture and in the community under the same environmental conditions. The communities were categorised into six interaction types, namely, competition, amensalism, parasitism, neutralism, commensalism and mutualism, based on a 10% difference in growth rates of the microbe in the co-culture and the monoculture (Heinken and Thiele 2015). The change in productivity in an organism in the community and the monoculture in various environments is collated in Figure 5. In a community with organisms A and B where

$$Mono_A + Mono_B = Comm_A Comm_B$$

$$\Delta Productivity_A = Productivity(Comm_A) - Productivity(Mono_A)$$

$$\Delta Productivity_B = Productivity(Comm_B) - Productivity(Mono_B)$$

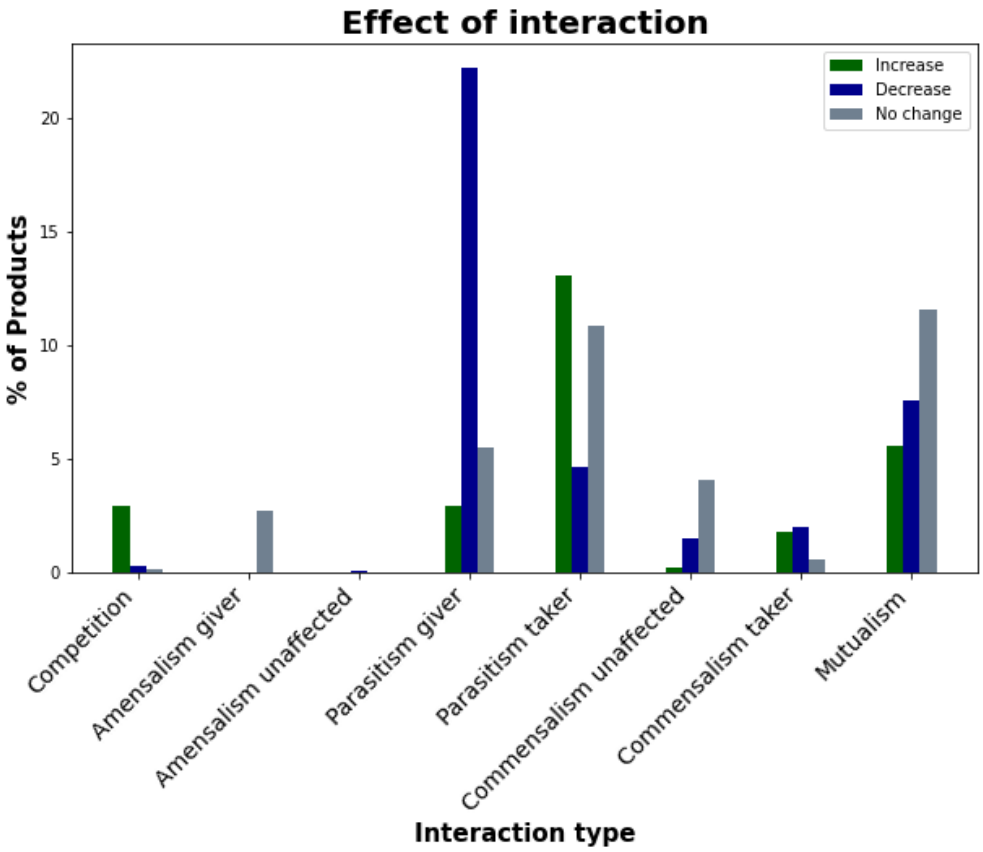

Fig 5. Effect of interaction type on the productivity of communities vs monocultures across environments. 'Increase' and 'Decrease' denotes the improvement or decline in the productivity of the given product in an organism in the community compared to that of the monocultures. 'No

change' represents no significant difference in productivity between communities and monocultures. Neutralism was not observed.

We observe that the organism with improved growth or a 'positive' interaction shows better production in the community when compared to the organism with decreased growth. This analysis of each partner in the community rather than the whole community helps us understand the effect of the interaction on each microbial partner. For example, 'parasitism taker' and 'parasitism giver' have varied effects where the 'giver', which has reduced growth, shows a decline in productivity, whereas the 'taker' with improved growth showcases enhanced productivity.

3.4 Tailored Metabolism: Communities and Monocultures Show Distinct Carbon Preferences

Another key factor we examined is the impact of different carbon sources on the growth and biosynthetic capabilities of microbial systems. In this analysis, we replaced the glucose in the 'rich' and 'minimal' media with 10 mmol/L of the carbon source under investigation. We tested seven different carbon sources and compared the productivity of communities and monocultures across all four environments, as shown in Figure 6.

Our findings show that while there are some variations, lactose and sucrose consistently enhance productivity in communities, whereas maltose and fructose favour monocultures. This insight can guide the design or supplementation of fermentation media: the choice of carbon source should align with the microbial system—or the microbial system should be selected based on the available carbon source.

To explore this further, we calculated the ratio of the change in productivity to the maximum productivity of monocultures ($Productivity\ ratio$) for four different products, as shown in Figure 7. Some communities, like *Shewanella oneidensis – Klebsiella pneumoniae*, showed minimal sensitivity to various carbon sources. However, others, such as *S. cerevisiae – L. lactis,* displayed varying productivity across carbon sources. In some cases, like *E. coli – S. cerevisiae*, the community does not grow under specific carbon sources. Moreover, this behaviour can also be product-dependent. Thus, product-specific analysis is crucial for identifying the optimal combination of the carbon source and microbial system.

$$Productivity\ ratio = \frac{\Delta Productivity}{abs\left(\max\left(Productivity(Mono_A), Productivity(Mono_B)\right)\right)}$$

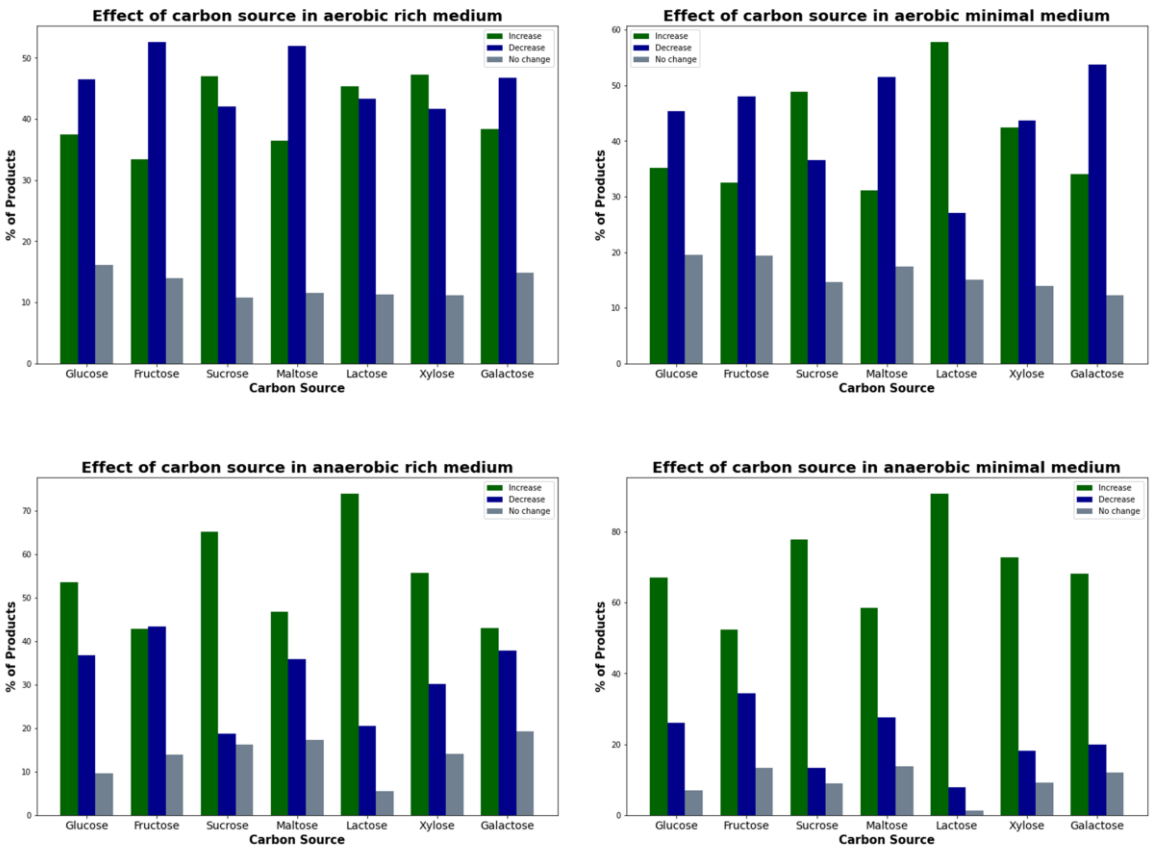

Fig 6. Effect of carbon source on the productivity of communities vs monocultures. The fraction of products showing increased or decreased productivity when switching from monocultures to communities is illustrated across different carbon sources. An 'Increase' indicates improved productivity in the community, while a 'Decrease' reflects a decline compared to monocultures. 'No change' signifies that the switch results in no significant difference in productivity.

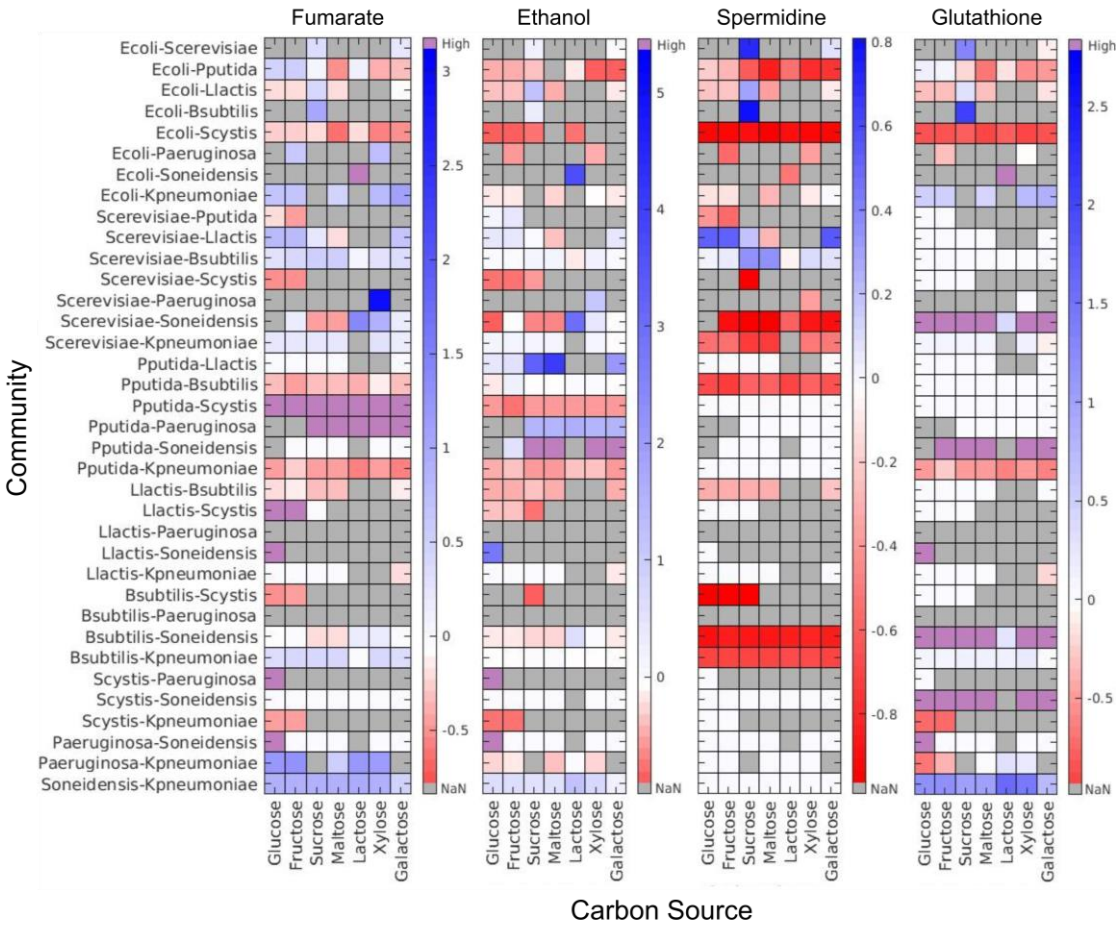

Fig 7. Effect of carbon source on the biosynthetic capability of different products in communities vs monocultures in the aerobic-rich environment. This figure illustrates the *Productivity ratio* of specific products across different communities under varying carbon sources. Positive values (blue) indicate an increase in the productivity of a given product in the community compared to the monocultures, while negative values (red) reflect a decline in productivity. If the product is only produced by the community and not by the monocultures, the value is shown in purple and labelled as 'High.' Conversely, when the community fails to grow on a specific carbon source, the value is represented in grey and marked as 'NaN.'

3.5 Computational Screening Reveals Ideal Microbial Systems Across Fermentation Conditions

While communities generally perform better in challenging environments, exceptions may occur. Therefore, it is essential to analyse the specific product of interest before selecting the most suitable microbial system. Furthermore, variations between communities mean that choosing the optimal community for a given nutrient source is crucial. Since experimentally comparing multiple communities is time-consuming and labour-intensive, computational analysis becomes essential.

342 We identified the best microbial systems for four key products from different classes
343 under minimal medium and anaerobic conditions, where many of the top-performing systems
344 were communities. The selection criteria need not be restricted to productivity but can also be
345 extended to other factors like abundance ratios. When two systems exhibit similar productivity,
346 choosing the one with a more balanced abundance may be preferable, as it can lead to a more
347 stable community. Moreover, the final choice can also be guided by biological insights, such as
348 ease of handling or specific biological characteristics that are desirable for the bioprocess of
349 interest. The optimal systems in the other environments are provided in Supplementary Files 2-
350 5.

351 Additionally, we compiled the top-performing systems for all 25 products across four
352 environments, as shown in Table 3. As expected from previous analyses, monocultures dominate
353 in the aerobic-rich medium, while communities excel in the anaerobic-minimal medium,
354 reinforcing the idea that harsher conditions favour biosynthesis in the communities. The detailed
355 results for all 25 products are listed in Supplementary Files 2-5. However, we would like to
356 highlight some interesting results obtained in Table 4. Interestingly, these correlate with the
357 findings of some experimental studies as listed in the references.

358 **Table 3. Best microbial system under different environments across all products**

#	Aerobic-rich		Aerobic-minimal		Anaerobic-rich		Anaerobic-minimal	
	Microbial system	**No. of Products**	**Microbial system**	**No. of Products**	**Microbial system**	**No. of Products**	**Microbial system**	**No. of Products**
1	*E. coli*	16	*E. coli*	15	*E. coli*	16	*E. coli - P. aeruginosa*	16
2	*K. pneumoniae*	14	*K. pneumoniae*	14	*E. coli - K. pneumoniae*	16	*E. coli*	15
3	*S. cerevisiae*	14	*S. cerevisiae - L. lactis*	14	*E. coli - S. oneidensis*	16	*B. subtilis - K. pneumoniae*	14
4	*S. cerevisiae - L. lactis*	14	*E. coli - P. aeruginosa*	13	*K. pneumoniae*	13	*E. coli - C. ljungdahlii*	14
5	*S. cerevisiae - K. pneumoniae*	13	*E. coli - K. pneumoniae*	12	*S. oneidensis - K. pneumoniae*	13	*P. aeruginosa - K. pneumoniae*	14
6	*S. oneidensis - K. pneumoniae*	13	*S. cerevisiae*	12	*B. subtilis*	11	*S. oneidensis - K. pneumoniae*	13
7	*B. subtilis*	12	*S. cerevisiae - P. aeruginosa*	12	*P. aeruginosa - S. oneidensis*	11	*K. pneumoniae*	12
8	*B. subtilis - K. pneumoniae*	11	*S. oneidensis - K. pneumoniae*	12	*B. subtilis - K. pneumoniae*	9	*P. aeruginosa - S. oneidensis*	11
9	*E. coli - K. pneumoniae*	11	*B. subtilis*	11	*C. ljungdahlii - B. subtilis*	8	*C. ljungdahlii - B. subtilis*	10
10	*P. aeruginosa - S. oneidensis*	11	*E. coli - P. putida*	10	*L. lactis - S. oneidensis*	8	*E. coli - S. oneidensis*	10

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Table 4. Results that align closely with experimental findings

#	Product	System Name	Productivity in Community (mmol/L/hr)	Productivity in Monoculture A (mmol/L/hr)	Productivity in Monoculture B (mmol/L/hr)	Improvement in productivity (%)	Abundance	Environment	Reference
1	1,3 - Propanediol	*S. oneidensis - K. pneumoniae*	0.064	0	0.0519	23.31	0.32,0.68	Anaerobic-rich	(Wang et al. 2023)
2		*P. aeruginosa - K. pneumoniae*	0.053	0	0.018	196.11	0.58,0.42	Anaerobic-minimal	(Li, Zhu, and Ge 2019)
3	2,3 - Butanediol	*P. aeruginosa - K. pneumoniae*	0.073	0	0.036	103.06	0.58,0.42	Anaerobic-minimal	(Li, Zhu, and Ge 2019)
4	Hydrogen	*C. ljungdahlii - B. subtilis*	0.684	0.091	0	647.92	0.87,0.13	Anaerobic-rich	(Chang et al. 2008)
5	Ethanol	*S. cerevisiae – B. subtilis*	0.630	0.416	0.604	4.41	0.69,0.31	Aerobic-rich	(Tantipaibulvut et al. 2015)
		S. cerevisiae - L. lactis	0.584	0.416	0.009	40.22	0.84,0.16	Aerobic-rich	(Carvalho et al. 2015)
		S. cerevisiae - L. lactis	0.410	0.363	0.008	13.03	0.65,0.35	Aerobic-minimal	(Li, Zhu, and Ge 2019)

3.6 Optimising Biomass Ratios Enhances Productivity in Microbial Communities

The initial biomass ratio of organisms within a community can significantly influence growth, abundance, and overall community dynamics. By adjusting the inoculum ratio, we can further enhance the biosynthetic capabilities of the community. Tables 5 and 6 illustrate how variations in inoculum ratios impact 1,3-propanediol (1,3-PDO) production in *P. aeruginosa* - *K. pneumoniae* and *S. oneidensis* - *K. pneumoniae* communities. Product concentrations vary with inoculum ratios, highlighting distinct responses between the two communities. While increasing *K. pneumoniae* inoculum enhances product titer in both, the *S. oneidensis* - *K. pneumoniae* community shows an increase in total biomass, whereas *P. aeruginosa* - *K. pneumoniae* displays a growth reduction. Moreover, higher *K. pneumoniae* inoculum in *S. oneidensis* - *K. pneumoniae* leads to a more skewed abundance ratio. These findings suggest that inoculum ratios can have varied effects on community dynamics and biosynthetic output, emphasizing the need for careful ratio optimization.

Table 5. Effect of inoculum ratio on the productivity of 1,3-PDO in *P. aeruginosa* - *K. pneumoniae* community

Biomass ratio	Biomass A (g/L)	Biomass B (g/L)	Total Biomass (g/L)	Abundance	Product concentration (mmol/L)
0.9,0.1	0.67	0.03	0.70	0.96,0.04	0.01
0.8,0.2	0.63	0.06	0.69	0.91,0.09	0.02
0.7,0.3	0.59	0.09	0.68	0.87,0.13	0.03
0.6,0.4	0.54	0.12	0.66	0.81,0.19	0.03
0.5,0.5	0.48	0.16	0.64	0.75,0.25	0.04
0.4,0.6	0.39	0.19	0.58	0.67,0.33	0.06
0.3,0.7	0.33	0.23	0.56	0.59,0.41	0.07
0.2,0.8	0.23	0.27	0.50	0.47,0.53	0.08
0.1,0.9	0.14	0.32	0.46	0.31,0.69	0.09

381 **Table 6. Effect of inoculum ratio on the productivity of 1,3-PDO in *S. oneidensis* - *K.***
382 ***pneumoniae* community**

Biomass ratio	Biomass A (g/L)	Biomass B (g/L)	Total biomass (g/L)	Abundance	Product concentration (mmol/L)
0.1,0.9	0.47	0.12	0.59	0.79,0.21	0.04
0.2,0.8	0.40	0.23	0.62	0.64,0.36	0.07
0.3,0.7	0.35	0.33	0.68	0.51,0.49	0.09
0.4,0.6	0.29	0.41	0.7	0.41,0.59	0.11
0.5,0.5	0.23	0.48	0.71	0.32,0.68	0.14
0.6,0.4	0.19	0.57	0.76	0.24,0.76	0.14
0.7,0.3	0.13	0.63	0.76	0.18,0.82	0.18
0.2,0.8	0.09	0.67	0.76	0.11,0.88	0.22
0.9,0.1	0.04	0.75	0.8	0.05,0.95	0.23

383 **3.7 Validation**

384 Though some of the results we obtain corroborate with experimental studies as listed in Table 4,
385 these studies only talk about the biosynthetic capability of the community and not the
386 monoculture. There are very few studies that compare the production capabilities of the
387 monoculture and the community in the same environment. In one such study, they evaluated the
388 production of 1,3-PDO in *Klebsiella pneumoniae* monoculture and the *Klebsiella pneumoniae* –
389 *Shewanella oneidensis* community using glycerol as the carbon source under anaerobic
390 conditions. They found that *Shewanella oneidensis* acts as an electron mediator in the co-culture
391 to attain better production of 1,3-PDO. Notably, under anaerobic conditions, this community is
392 the top producer of 1,3-PDO in our analysis. To demonstrate the reliability of COSMOS, we
393 simulated the growth of the community and the monoculture in the medium used in the
394 experimental study and compared the results. The first analysis is to identify the optimum
395 glycerol concentration for the monoculture. We found 50 g/L glycerol to be the optimum
396 concentration for 1,3-PDO production, which correlates with the experiment values, as shown in
397 Table 7.
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399
400
401

Table 7. Concentration of 1,3 propanediol in experiments and simulations under varying glycerol concentration

Glycerol concentration (g/L)	Concentration of 1,3 PDO in experiments (g/L)	Concentration of 1,3 PDO in COSMOS (g/L)
40	11.3	1.76
50	12.68	1.83
70	12.21	1.58

For the community analysis, the experimental group used a fed-batch reactor under anaerobic conditions where the initial glycerol concentration was 50 g/L. When the glycerol concentration falls below 10 g/L, additional glycerol is introduced to maintain a final concentration of 30 g/L. They found that the community produced 32.01 g/L of 1,3-PDO, which was significantly higher when compared to the monoculture. They also optimised the inoculum ratio and found that a 1:1 ratio of *Klebsiella pneumoniae*: *Shewanella oneidensis* resulted in the highest product concentration, though the exact values are not listed. We did a similar analysis using COSMOS and found that the community was, in fact, able to produce more product in the fed-batch system when compared to the monoculture. However, we found that both the inoculum ratios of 1:1 and 2:1 performed equally well, as shown in Table 8. This might be because we have used the wild-type models of both organisms and lack the strain-specific information that might be required.

Table 8. Concentration of 1,3 -propanediol with varying inoculum ratio according to simulations

Initial biomass ratio	1,3 Propanediol (g/L)	Abundance
2,1	2.42	0.04,0.96
1,1	2.20	0.07,0.93
1,2	0.31	0.1,0.9
1,3	0.28	0.12,0.88

The study reported monoculture and co-culture yields of 0.4 g/g and 0.44 g/g, respectively, reflecting a 10% improvement. COSMOS predicted yields of 1.50 g/g for monocultures and 1.33 g/g for co-cultures, indicating a 12.6% increase. Although the absolute values differ, the algorithm effectively captured the trend in performance, identifying optimal substrate concentrations and inoculum ratios. This demonstrates the potential of dynamic modelling in bioprocess design, offering a powerful tool for efficiently comparing multiple communities and monocultures to identify the most productive configurations.

4 Discussion

Communities often demonstrate superior biosynthetic capability compared to monocultures (Rapp, Jenkins, and Betenbaugh 2020; Xu and Tschirner 2011). However, their application in bioproduction remains limited to cases where their productivity is already known or where their metabolic diversity is essential, such as when one community member can metabolise a complex carbon source that the others cannot. Studies that directly compare the biosynthetic potential of multiple communities remain scarce (Wilken et al. 2018), leaving a gap in identifying the most effective microbial systems.

In contrast to tools such as FLYCOP, which focuses on optimising a single co-culture, COSMOS directly compares monocultures and communities under identical environmental conditions. By integrating dFBA and FVA, COSMOS offers more granular insights into system performance across a wider range of environmental conditions. This approach equips researchers to choose optimal microbial systems for specific products or feedstocks.

This choice between a community and a monoculture primarily hinges on their relative productivity. If a community offers substantially higher productivity, it is the better option for bioproduction. However, when productivity is comparable, monocultures are preferred for their ease of control and simplicity. In cases where operational simplicity takes precedence over maximum output, slightly less productive monocultures can be selected. COSMOS streamlines this decision-making process by assessing the biosynthetic potential of diverse microbial systems under identical conditions. To simulate microbial growth of such synthetic consortia, we employed parsimonious dynamic FBA that can handle fluctuating growth rates. Unlike steady-state modelling, which assumes uniform time-averaged growth rates across all organisms, dFBA captures organism-specific differences in biosynthetic capabilities without relying on detailed kinetic data. We paired this with FVA to address the inherent redundancy in FBA solutions, ensuring reliable comparisons of productivity across systems by computing maximum fluxes through product exchange reactions at each time step.

We tested COSMOS across four environments: aerobic-rich, aerobic-minimal, anaerobic-rich, and anaerobic-minimal media. While overall productivity was highest in aerobic nutrient-rich conditions, communities performed significantly better in anaerobic, nutrient-limited environments. This phenomenon is likely due to the cooperative interactions within communities, which enhance growth and survival under stressful conditions. These findings suggest that communities may be more suitable for bioprocesses involving nutrient-poor substrates, such as agricultural residues, wastewater, or anaerobic sludge digestion. COSMOS also revealed that mutualistic and commensal interactions become more prevalent under harsh conditions, further improving community productivity.

Interestingly, exceptions were observed, where a community may excel in nutrient-rich conditions, while a monoculture might outperform in nutrient-poor settings, depending on the specific product being targeted. This underscores the need for the systematic evaluation of both communities and monocultures before choosing the microbial system for a specific product. COSMOS helps us maximise productivity in the chosen fermentation medium. Moreover, identifying positive microbial interactions can inform better community design for biomanufacturing, optimizing both productivity and stability.

We also examined the effect of carbon sources on these systems. Certain carbon sources, like lactose and sucrose, enable better productivity in the community, while maltose and fructose boost monoculture performance. This analysis aids in selecting or enriching fermentation media based on specific carbon sources. The algorithm can also be easily extended to study the effect of two or more carbon sources. COSMOS also equips us to optimise the initial inoculum ratio to further improve productivity.

We have demonstrated that COSMOS is able to successfully capture the effect of carbon source concentration and inoculum ratio in the *Klebsiella pneumoniae* – *Shewanella oneidensis* community and monocultures. The trends in yield and productivity under different conditions were consistent with the experimental study, demonstrating the reliability of the algorithm. Furthermore, many communities identified through our analysis have been found to form stable consortia in experimental studies, with some already proving their biosynthetic potential (as listed in Table 4).

Notably, the insights from this study have direct relevance to fields like biofuel production, wastewater treatment, and pharmaceutical biosynthesis. Moreover, by optimising microbial systems for nutrient-limited and waste-derived feedstocks, our approach aligns with the UN Sustainable Development Goals (SDGs), particularly SDG 9 (Industry, Innovation, and Infrastructure) and SDG 12 (Responsible Consumption and Production). In summary, COSMOS provides a robust computational framework to evaluate and compare the productivity of monocultures and microbial communities, addressing the challenges of experimental testing. It offers insights into how environmental factors, carbon sources, and inoculum ratios affect performance, helping researchers make well-informed decisions about which microbial system to use. Whether the goal is to work with nutrient-dense or nutrient-limited media, our approach ensures the selection of the most effective system, enhancing productivity, process efficiency, and community stability.

5 Conclusion

As industries pivot toward sustainable practices, bioresources like agricultural residues and wastewater will play a vital role in producing specialty and bulk chemicals. Although

nutrient-rich media remain standard for high-value fermentation, optimizing nutrient-poor media is increasingly crucial for achieving net-zero carbon goals. However, the limited economic feasibility and lack of robust tools have hindered their widespread adoption.

Our study demonstrates that *in silico* analyses can effectively optimise microbial systems to enhance productivity, especially in challenging environments. Microbial communities, for instance, are better suited for harsh conditions—even with simple carbon sources—thanks to their complementary metabolism and cooperative interactions. However, some monocultures can still outperform communities, highlighting the need for systematic computational screening to identify the most promising candidates before advancing to laboratory testing. As more models and kinetic data become available, the predictive power of algorithms will improve, further advancing bioprocess design.

Ultimately, this approach supports the rational design of microbial systems, advancing synthetic biology and sustainable biomanufacturing. By understanding when to switch between monocultures and communities and identifying key environmental and metabolic factors influencing performance, we can develop more robust and efficient microbial systems. We believe that our framework and such computational strategies will be key enablers in the transition to circular bio-economies.

Acknowledgements

LR acknowledges the fellowship from the Ministry of Education, Government of India. KR acknowledges support from the Science and Engineering Research Board (SERB) MATRICS Grant MTR/2020/000490, IIT Madras, Centre for Integrative Biology and Systems mEdicine (IBSE) and the Wadhwani School of Data Science and AI.

Data Availability

All models and analysis scripts used in this study are openly available at https://github.com/RamanLab/COSMOS

Author Contributions

Lavanya Raajaraam: Conceptualization, Performing Experiments, Analysis of Results, Writing - original draft. Karthik Raman: Conceptualisation, Analysis of Results.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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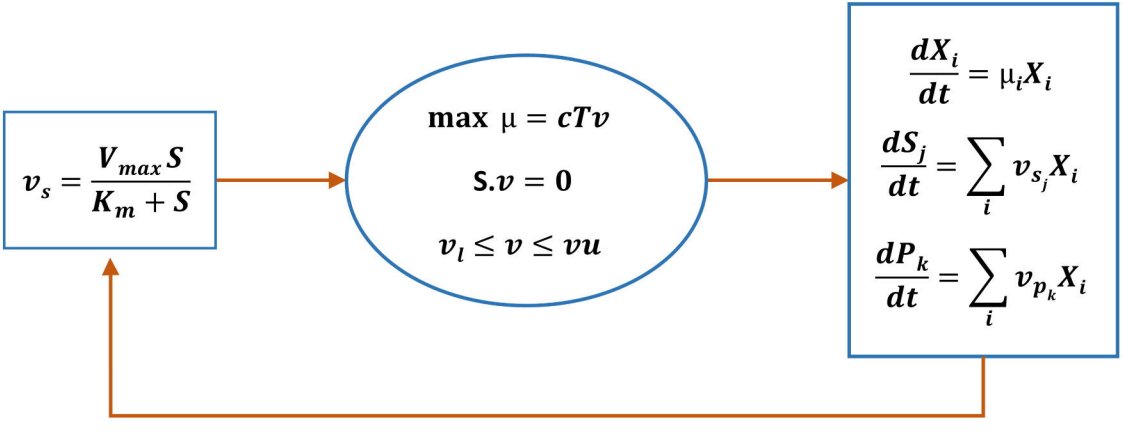
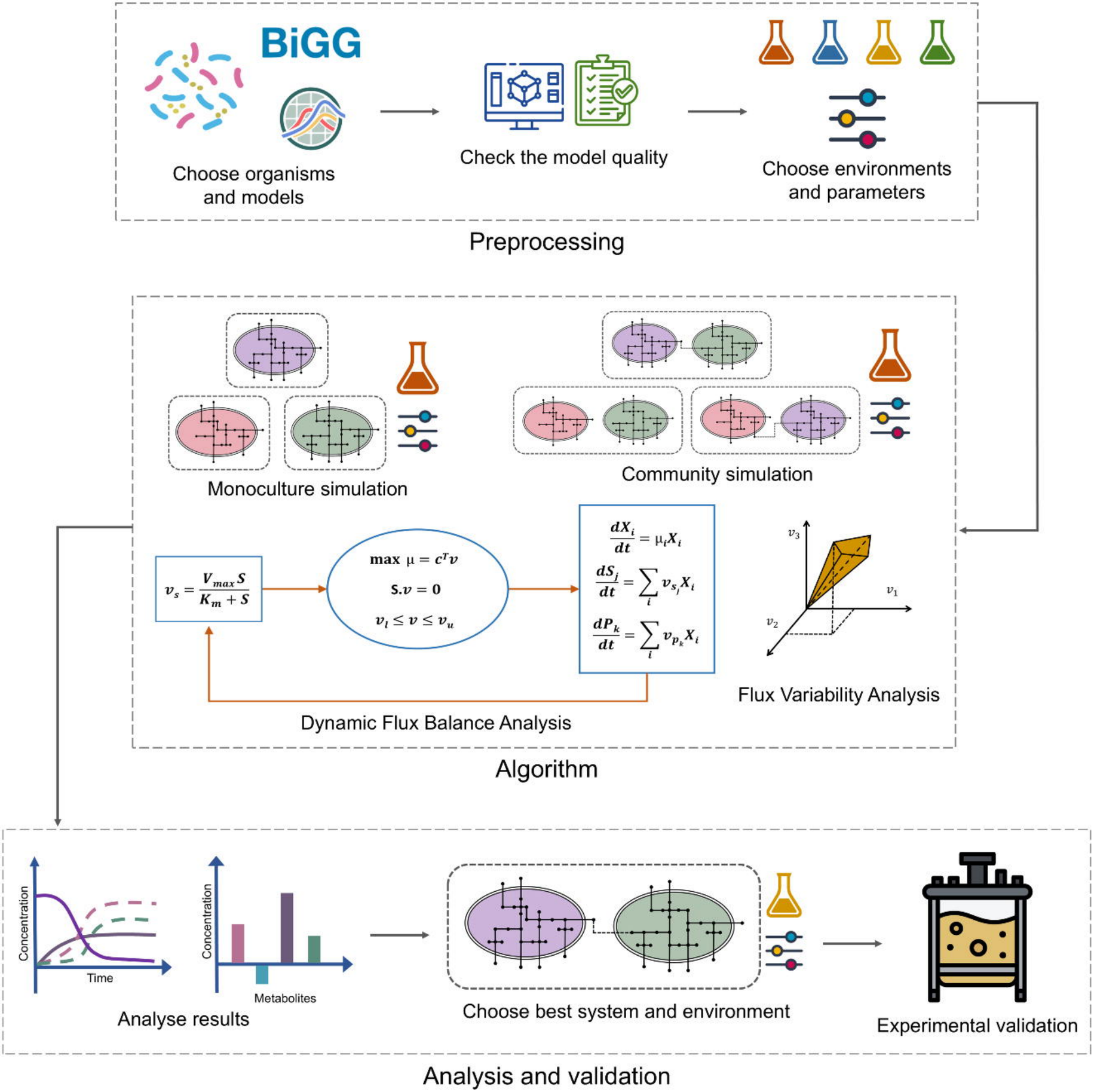
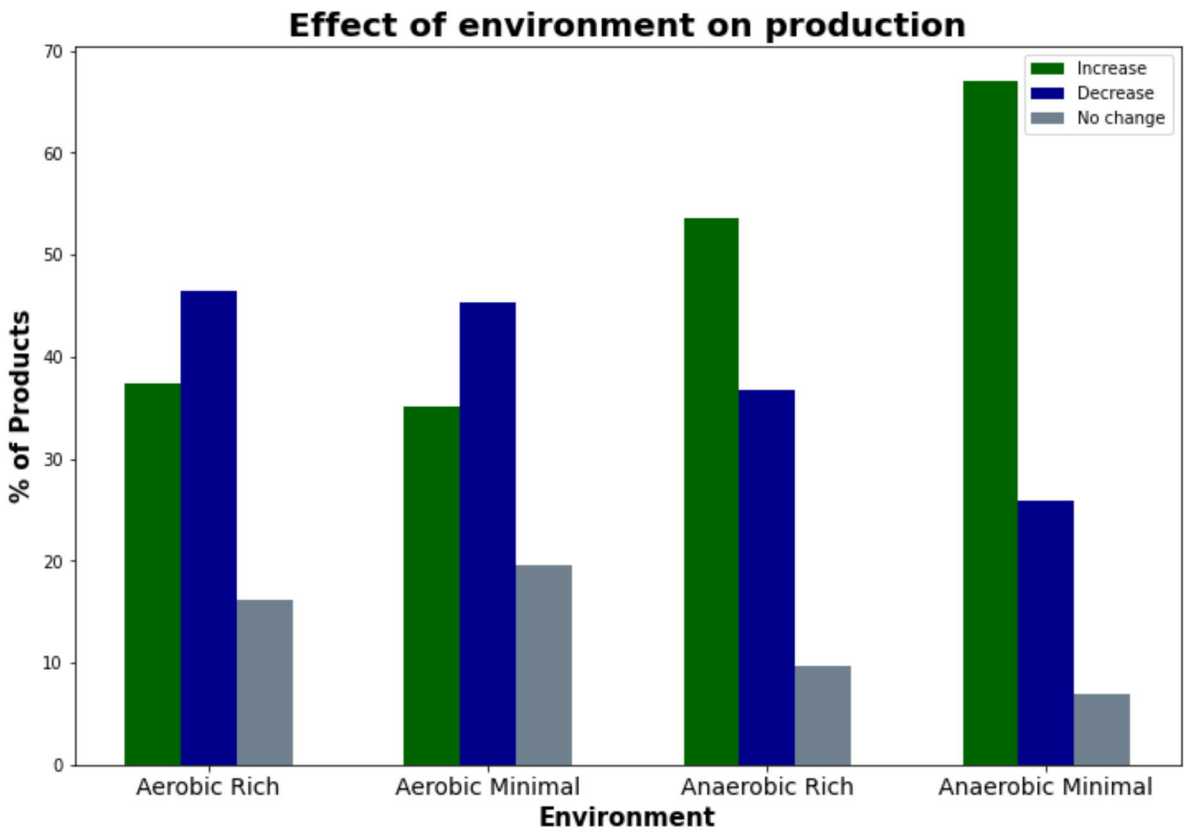
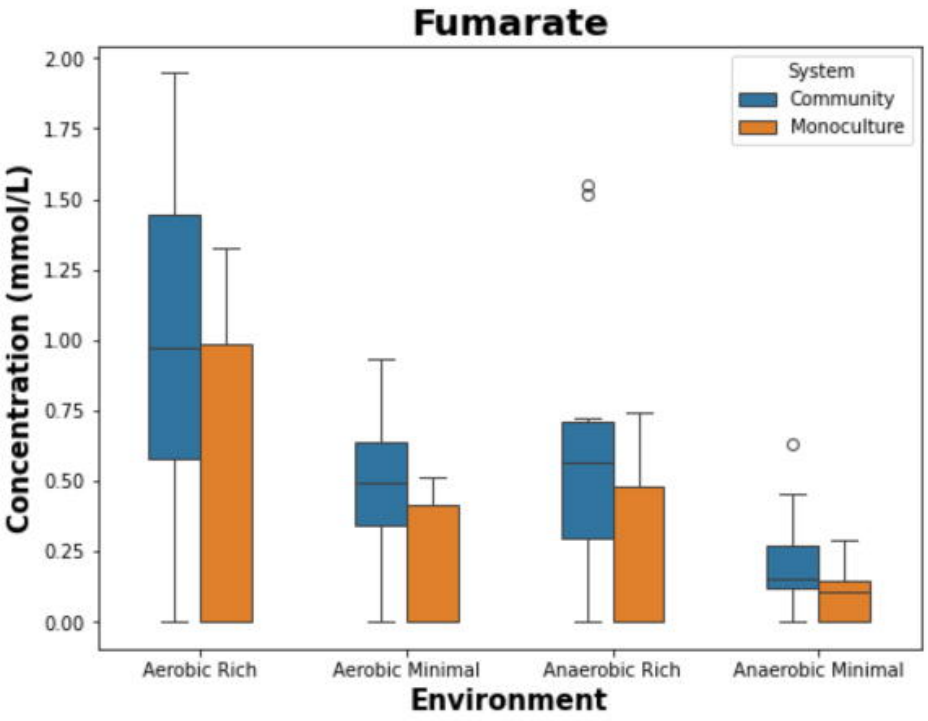
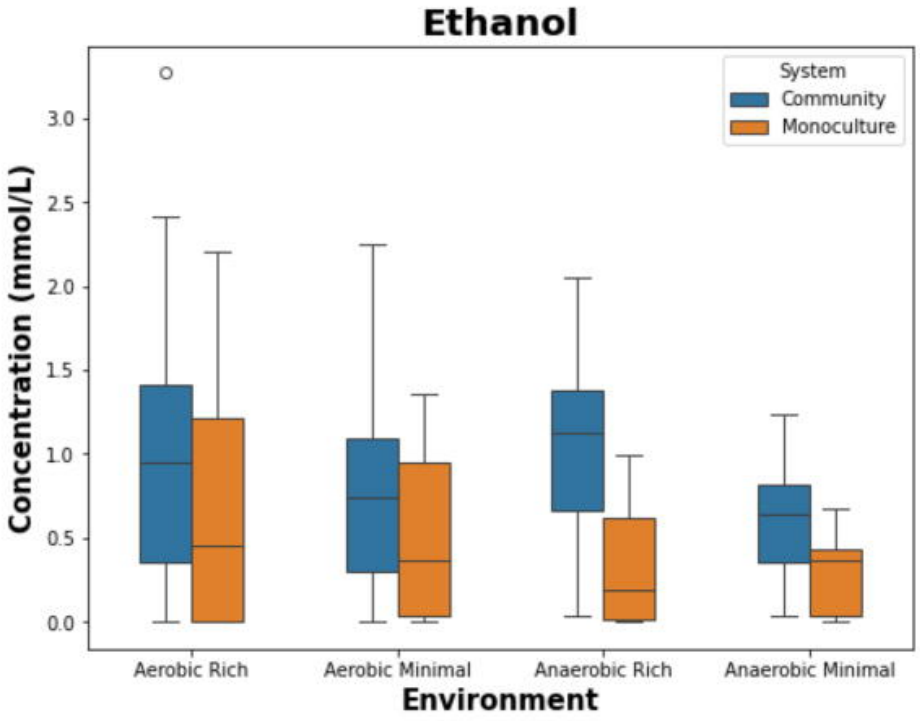
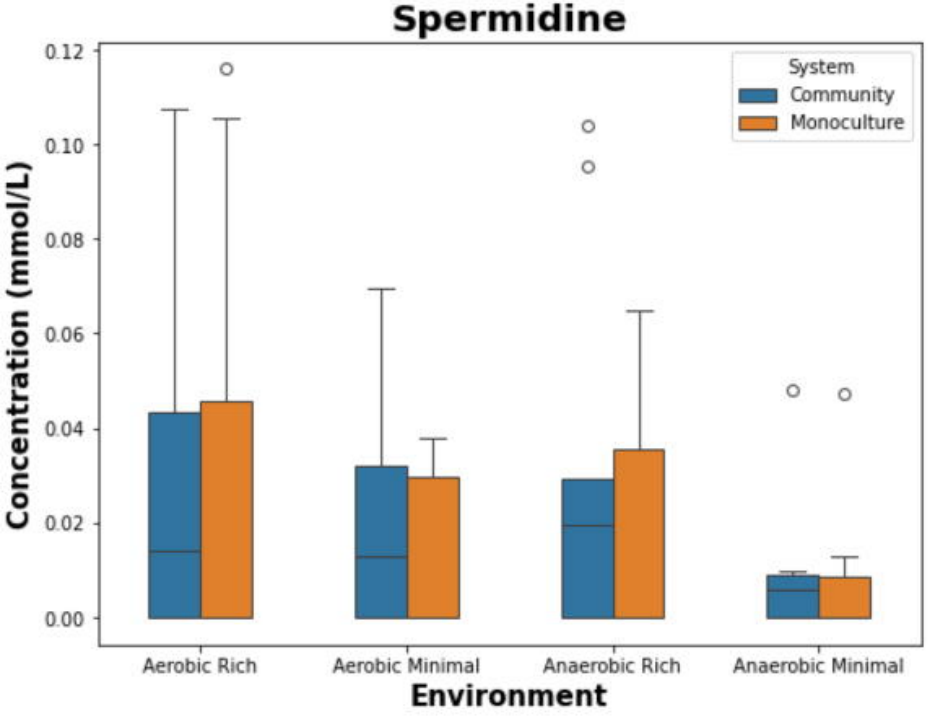

$v_s = \frac{V_{max} S}{K_m + S}$
max $\mu = cTv$
$S.v = 0$
$v_l \leq v \leq vu$
$\frac{dX_i}{dt} = \mu_i X_i$
$\frac{dS_j}{dt} = \sum_i v_{s_j} X_i$
$\frac{dP_k}{dt} = \sum_i v_{p_k} X_i$

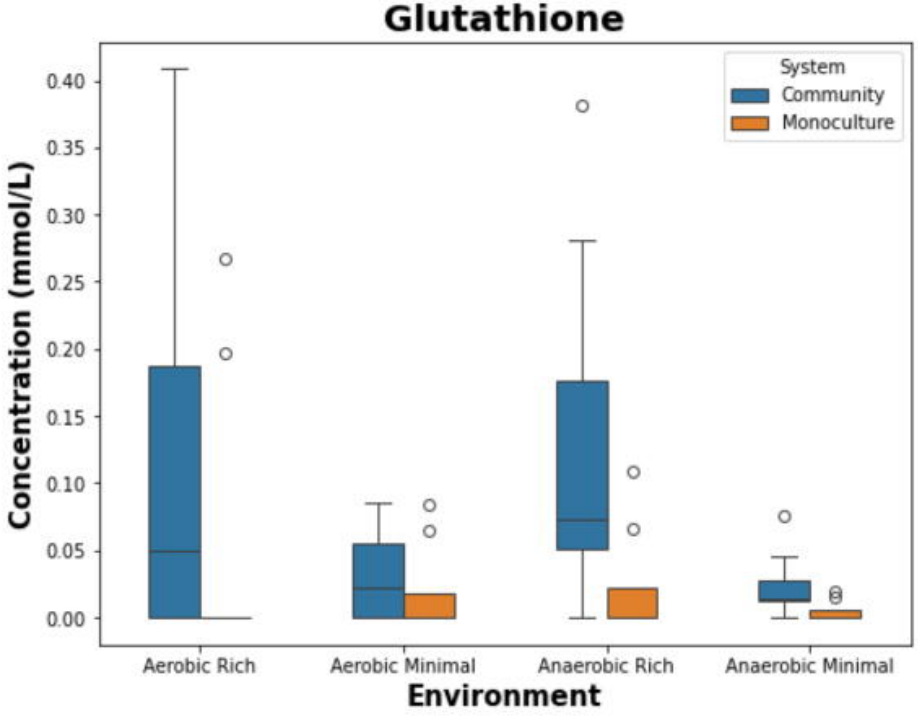

BiGG
Choose organisms and models
Check the model quality
Choose environments and parameters
Preprocessing
Monoculture simulation
Community simulation
$v_s = \frac{V_{max} S}{K_m + S}$
$\max \mu = c^T v$
$S.v = 0$
$v_l \leq v \leq v_u$
$\frac{dX_i}{dt} = \mu_i X_i$
$\frac{dS_j}{dt} = \sum_i v_{s_j} X_i$
$\frac{dP_k}{dt} = \sum_i v_{p_k} X_i$
v_3
v_1
v_2
Flux Variability Analysis
Dynamic Flux Balance Analysis
Algorithm
Concentration
Time
Concentration
Metabolites
Analyse results
Choose best system and environment
Experimental validation
Analysis and validation

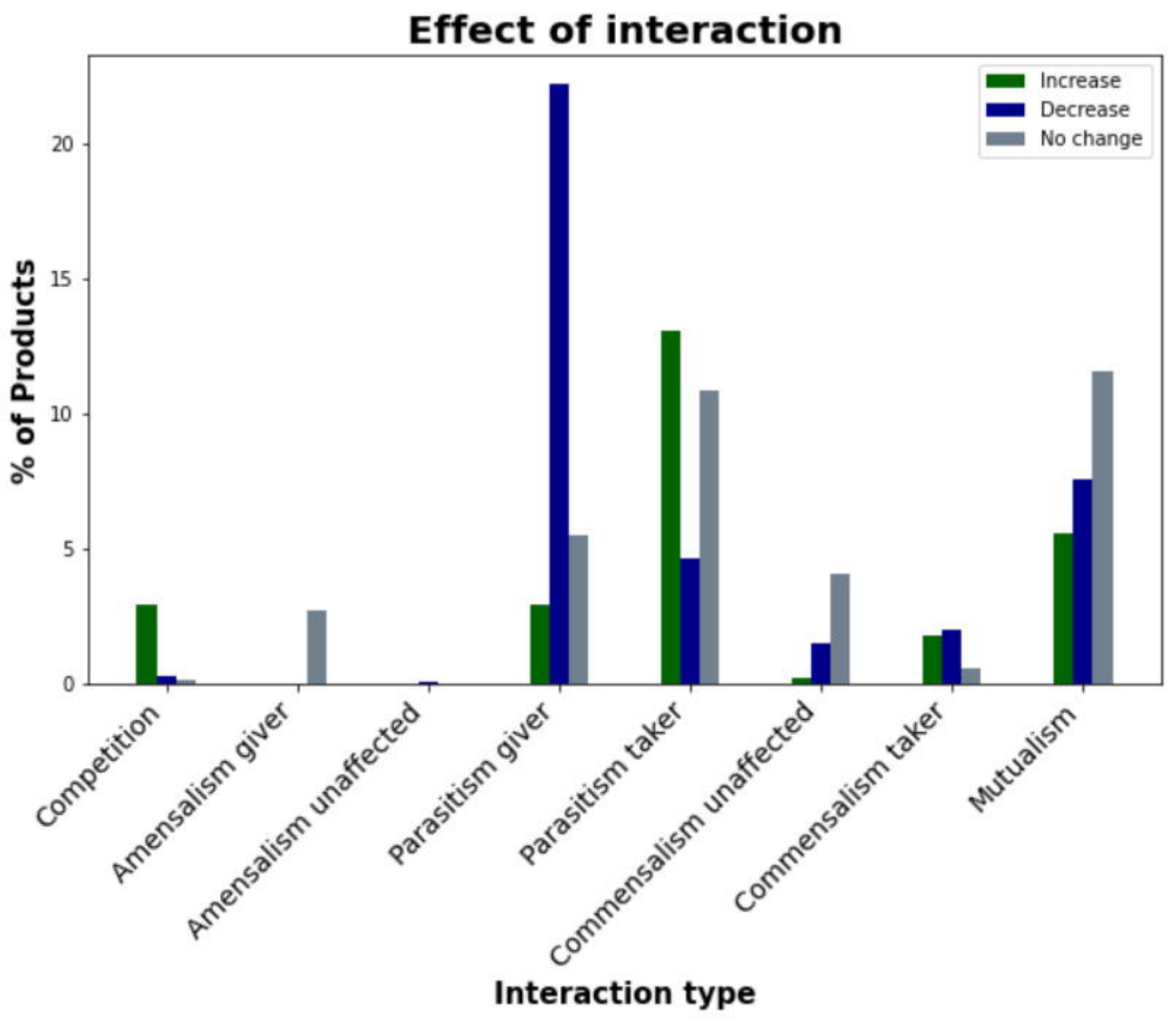

Effect of environment on production
Increase
Decrease
No change
% of Products
0
10
20
30
40
50
60
70
Aerobic Rich
Aerobic Minimal
Anaerobic Rich
Anaerobic Minimal
Environment

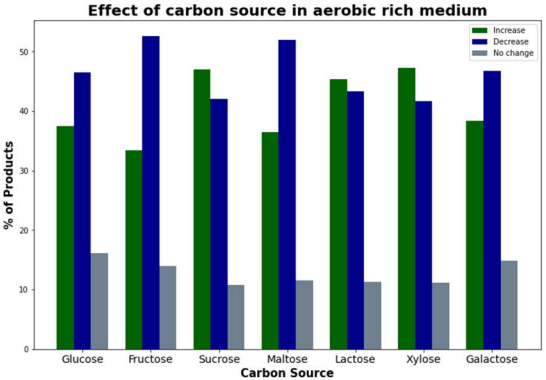

Fumarate
System
Community
Monoculture
Concentration (mmol/L)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Aerobic Rich
Aerobic Minimal
Anaerobic Rich
Anaerobic Minimal
Environment

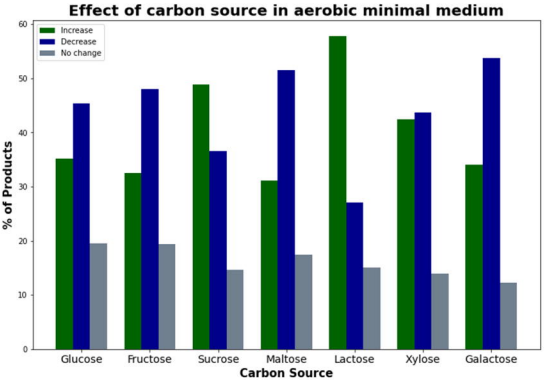

Ethanol
System
Community
Monoculture
Concentration (mmol/L)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Aerobic Rich
Aerobic Minimal
Anaerobic Rich
Anaerobic Minimal
Environment

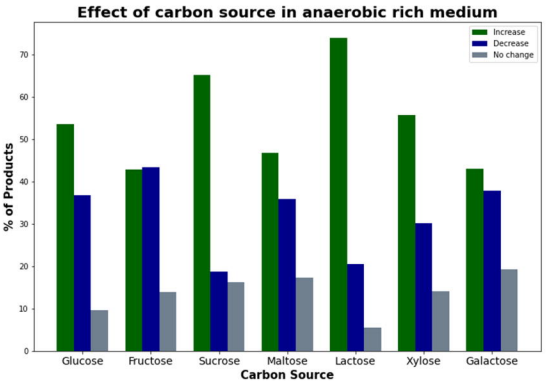

Spermidine
System
Community
Monoculture
Concentration (mmol/L)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Aerobic Rich
Aerobic Minimal
Anaerobic Rich
Anaerobic Minimal
Environment

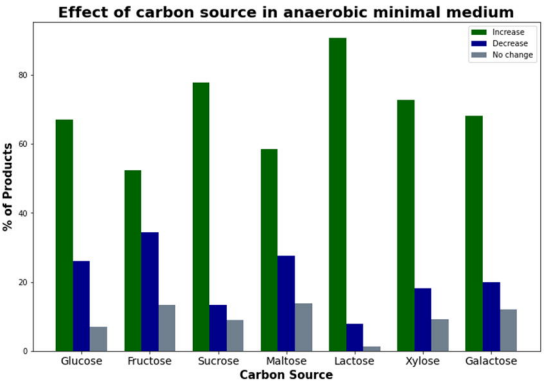

Glutathione
System
Community
Monoculture
Concentration (mmol/L)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Aerobic Rich
Aerobic Minimal
Anaerobic Rich
Anaerobic Minimal
Environment

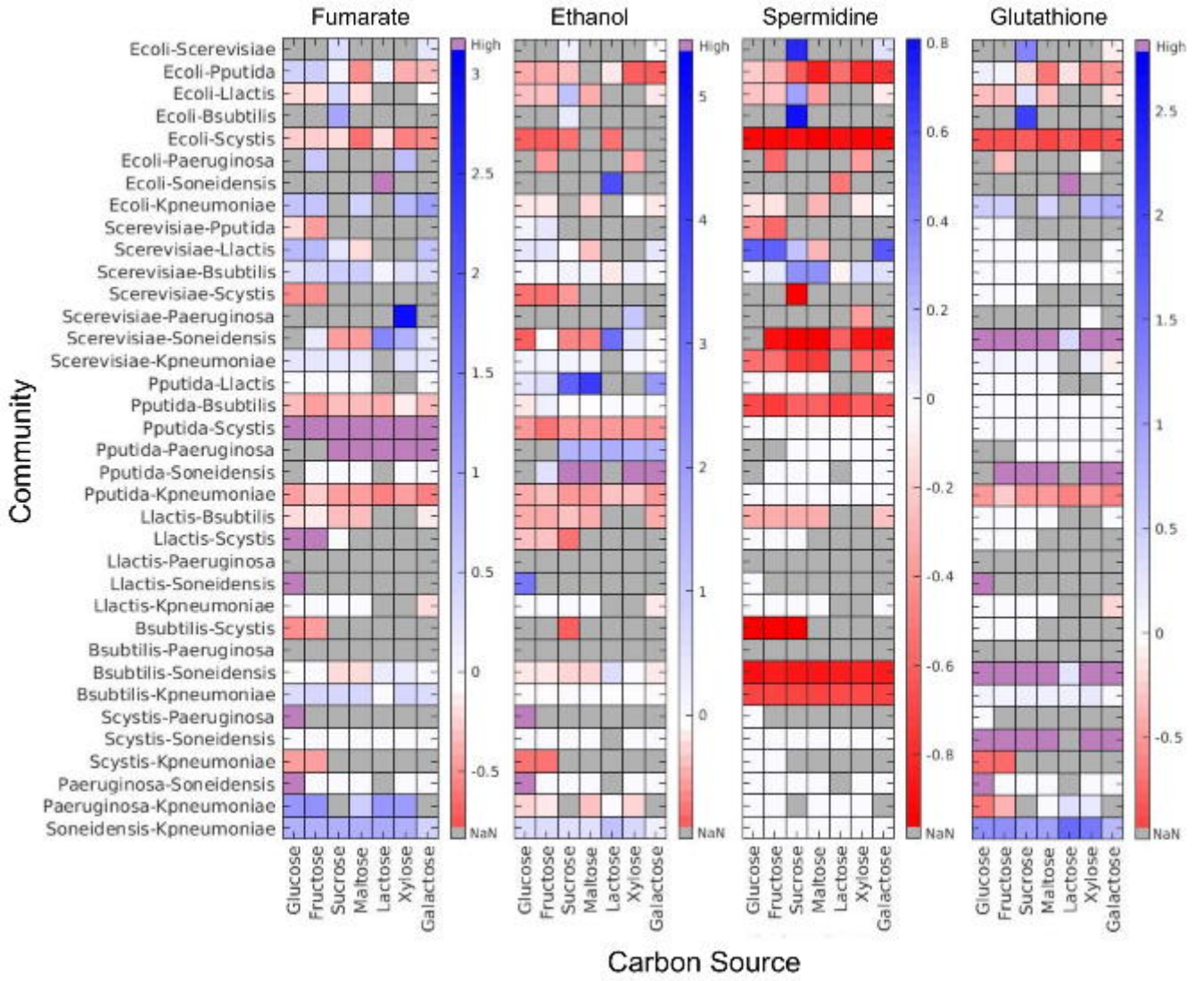

Effect of interaction
Increase
Decrease
No change
% of Products
0
5
10
15
20
Competition
Amensalism giver
Amensalism unaffected
Parasitism giver
Parasitism taker
Commensalism unaffected
Commensalism taker
Mutualism
Interaction type


Effect of carbon source in aerobic rich medium
Increase
Decrease
No change
% of Products
0
10
20
30
40
50
Glucose
Fructose
Sucrose
Maltose
Lactose
Xylose
Galactose
Carbon Source


Effect of carbon source in aerobic minimal medium
Increase
Decrease
No change
% of Products
0
10
20
30
40
50
60
Glucose
Fructose
Sucrose
Maltose
Lactose
Xylose
Galactose
Carbon Source


Effect of carbon source in anaerobic rich medium
Increase
Decrease
No change
% of Products
0
10
20
30
40
50
60
70
Glucose
Fructose
Sucrose
Maltose
Lactose
Xylose
Galactose
Carbon Source


Effect of carbon source in anaerobic minimal medium
Increase
Decrease
No change
% of Products
0
20
40
60
80
Glucose
Fructose
Sucrose
Maltose
Lactose
Xylose
Galactose
Carbon Source


Fumarate
Ethanol
Spermidine
Glutathione
Community
Carbon Source
Ecoli-Scerevisiae
Ecoli-Pputida
Ecoli-Llactis
Ecoli-Bsubtilis
Ecoli-Scystis
Ecoli-Paeruginosa
Ecoli-Soneidensis
Ecoli-Kpneumoniae
Scerevisiae-Pputida
Scerevisiae-Llactis
Scerevisiae-Bsubtilis
Scerevisiae-Scystis
Scerevisiae-Paeruginosa
Scerevisiae-Soneidensis
Scerevisiae-Kpneumoniae
Pputida-Llactis
Pputida-Bsubtilis
Pputida-Scystis
Pputida-Paeruginosa
Pputida-Soneidensis
Pputida-Kpneumoniae
Llactis-Bsubtilis
Llactis-Scystis
Llactis-Paeruginosa
Llactis-Soneidensis
Llactis-Kpneumoniae
Bsubtilis-Scystis
Bsubtilis-Paeruginosa
Bsubtilis-Soneidensis
Bsubtilis-Kpneumoniae
Scystis-Paeruginosa
Scystis-Soneidensis
Scystis-Kpneumoniae
Paeruginosa-Soneidensis
Paeruginosa-Kpneumoniae
Soneidensis-Kpneumoniae
Glucose
Fructose
Sucrose
Maltose
Lactose
Xylose
Galactose
High
NaN