MICROBIAL BIOTECHNOLOGY

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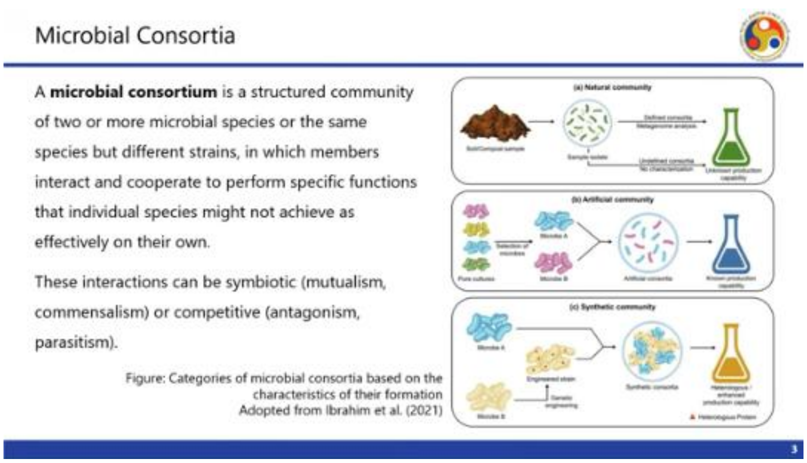
Lecture-22

Lec 22: Engineered Microbial Consortia for Industry

Hello friends, welcome to my course on microbial biotechnology. We are in module 6, discussing the industrial and pharmaceutical applications of microorganisms. In this lecture, we will discuss engineered microbial consortia for industry. This lecture is divided into three broad sections. In section 1, we will first discuss what a microbial consortium is, then we will discuss the importance of such microbial consortia,

and we will discuss the interactions between various strains in a consortium, the special interactions in microbial consortia, the types of microbial consortia, natural versus synthetic consortia, then the advantages of microbial consortia, the synergistic interactions, resource sharing, and the challenges in engineering microbial consortia, the stability, communication, and control, comparison with monoculture systems, advantages and limitations, methods to reconstruct synthetic consortia, strategy, based on rational design, adaptive evolution, comparison of the adaptive evolution strategies. Then we will also discuss the methods to construct synthetic consortia, source-based, top-down approach, bottom-up approach, and comparison of top-down and bottom-up approaches. We'll come to the details of the other two sections as we proceed. First, let us see what the idea of a microbial consortium is.

A microbial consortium is a structured community of two or more microbial species or the same species but with different strains, in which members interact and cooperate to perform specific functions that individual species might not achieve as effectively on their own. These interactions can be symbiotic, mutualistic, commensalistic, or competitive, antagonistic, or parasitic. So, here we can see the categories of microbial consortia based on the characteristics of their formation. The first one is the natural community, which we isolate from soil or, for example, from compost, and from there we can obtain the defined consortia whereby we do the metagenome analysis and then, in other cases, do not do any characterization.



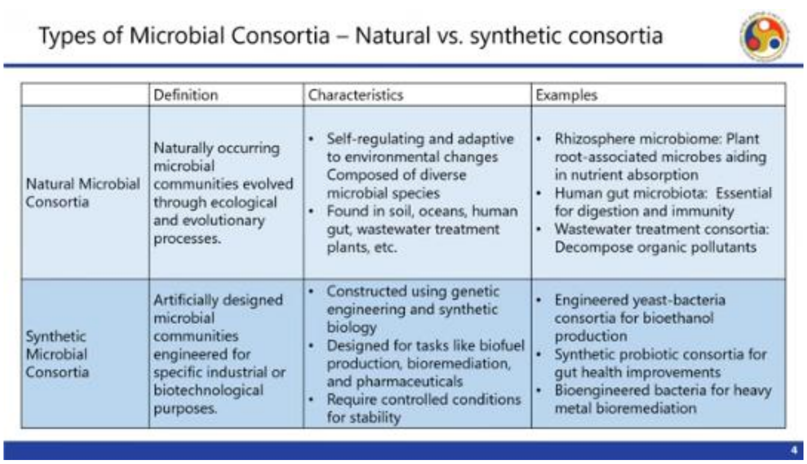
So, we use the undefined consortia. So, here the production capacity of such undefined consortia will be unknown. And in the artificial community, we have pure cultures. You can see different colors depicting different cultures or maybe different strains. From there, we select, say, two microbes A and B or two strains and then we constitute them into a consortia, and we know the production capability of these artificial consortia in advance.

Then we have a synthetic community where we have a microbe A and a microbe B. The microbe B is changed by genetic engineering. Maybe we have transferred some gene over there which produces a heterologous protein. And then we mix both the natural microbial strain A and the engineered microbial strain B and then constitute the consortia. Here, the production capability is enhanced due to this genetic engineering step taken in the development of the consortia. So, to sum up, we can have a natural community, an artificial community, and a synthetic community.

Now, based on the above discussion, we can see that there is a natural and synthetic consortia. In the natural microbial consortia, These communities evolve through ecological and evolutionary processes. In the synthetic microbial consortia, these are artificially designed microbial communities engineered for specific industrial or biotechnological purposes. In the first case, they are self-regulating and adaptive to environmental changes, composed of diverse microbial

species found in soil, oceans, human gut, wastewater treatment plants, etc. Some examples are the rhizosphere microbiome, a plant-road associated microbes aiding in nutrient absorption. Then we have the human gut microbiota essential for digestion and immunity. Then we have the wastewater treatment consortia which decompose organic pollutants. In the case of synthetic microbial consortia,

These are constructed using genetic engineering and synthetic biology. These are designed for tasks like biofuel production, bioremediation, pharmaceuticals. They require control conditions for stability. Examples include engineered yeast bacteria consortia for bioethanol production, synthetic probiotic consortia for gut health improvements, bioengineered bacteria for heavy metal bioremediation. What is the importance of microbial consortia?



Types of Microbial Consortia – Natural vs. synthetic consortia

	Definition	Characteristics	Examples
Natural Microbial Consortia	Naturally occurring microbial communities evolved through ecological and evolutionary processes.	• Self-regulating and adaptive to environmental changes Composed of diverse microbial species • Found in soil, oceans, human gut, wastewater treatment plants, etc.	• Rhizosphere microbiome: Plant root-associated microbes aiding in nutrient absorption • Human gut microbiota: Essential for digestion and immunity • Wastewater treatment consortia: Decompose organic pollutants
Synthetic Microbial Consortia	Artificially designed microbial communities engineered for specific industrial or biotechnological purposes.	• Constructed using genetic engineering and synthetic biology • Designed for tasks like biofuel production, bioremediation, and pharmaceuticals • Require controlled conditions for stability	• Engineered yeast-bacteria consortia for bioethanol production • Synthetic probiotic consortia for gut health improvements • Bioengineered bacteria for heavy metal bioremediation

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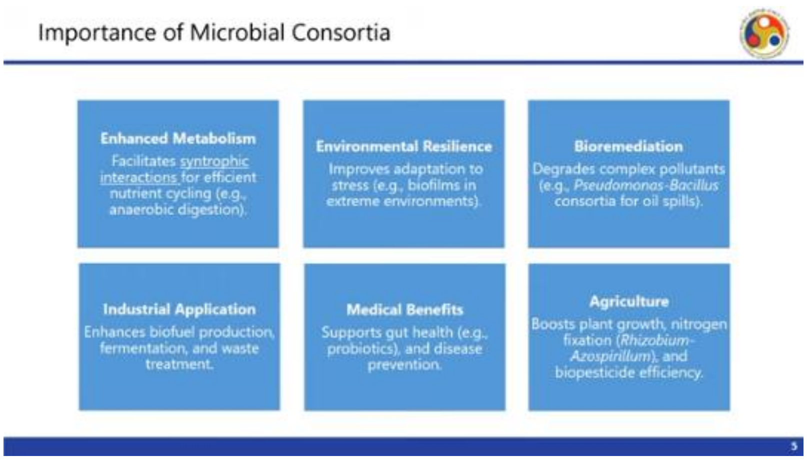
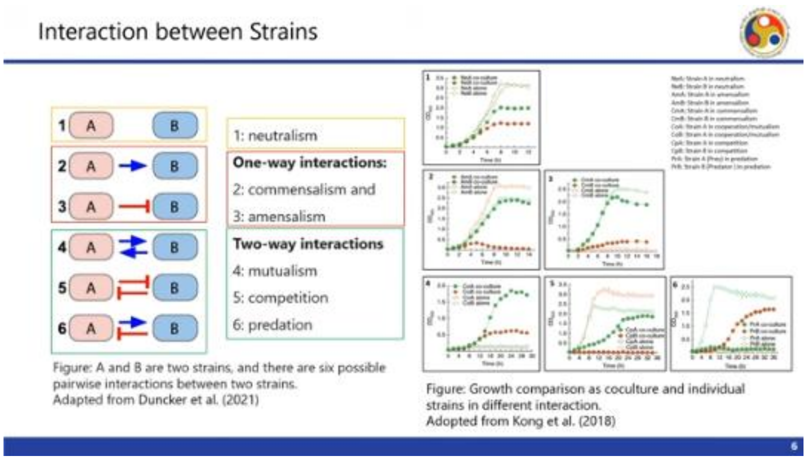
They provide enhanced metabolism. Such consortia facilitates syntropic interactions for efficient nutrient cycling, for example, anaerobic digestion. They provide environmental resilience, improves adaptation to stress, for example, biofilms in extreme environments. They can be used for bioremediation, degrades complex pollutants, e.g. Pseudomonas bacillus consortia for the oil spills.

These consortia have industrial applications whereby they enhance biofuel production, fermentation, and waste treatment. They also provide medical benefits, e.g., supporting gut health, e.g., probiotics and disease prevention. They are also useful in agriculture, where they boost plant growth, nitrogen fixation (for example, rhizobium and azospirillium), and biopesticide efficiency.



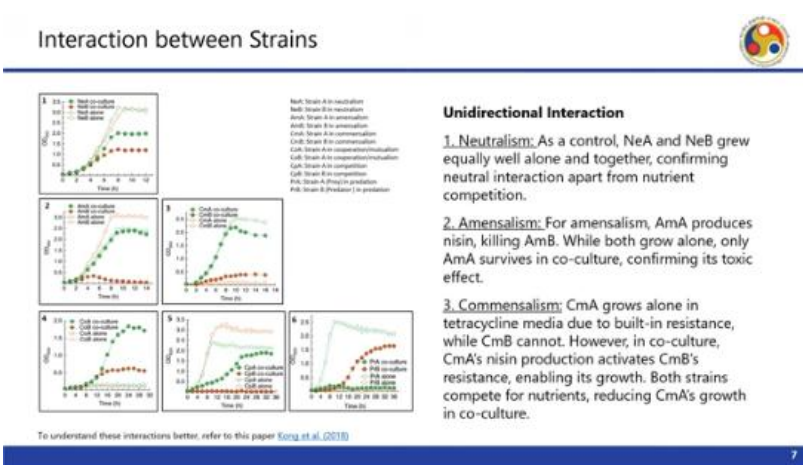
So, once we constitute these microbial consortia, or we put two microbes of different species or different strains together, what could be the interactions between these two entities? So, number one. There may be no interaction between them, which we call neutralism. Then there may be one-way interactions where A impacts B. That's commensalism, and it may also lead to amensalism. Then there may be two-way interactions where, in the case, both A and B influence each other or interact with each other in a positive way.

So that's mutualism. There may be competition between them, or in another case, one species may benefit the other, but the other may actually predate on it—that's predation. So in this, you can see the various interactions between the strains. In one, you can see there is a strain A and in neutralism, the growth kinetics are different when they are co-cultured and when they are cultured alone. For example, in this case, when NEA is co-cultured, here the strain is growing at a much lower rate than it used to grow when it is grown alone.



So, this is some kind of neutralism. As a control, NeA and NeB grew equally well alone and together, confirming neutral interaction apart from the nutrient competition. In the case of amensalism, in number two, you can see AmA produces nisin, killing AmB, while both grow alone. Only AmA survives in co-culture, confirming its toxic effect. Then comes the third relationship, commensalism.

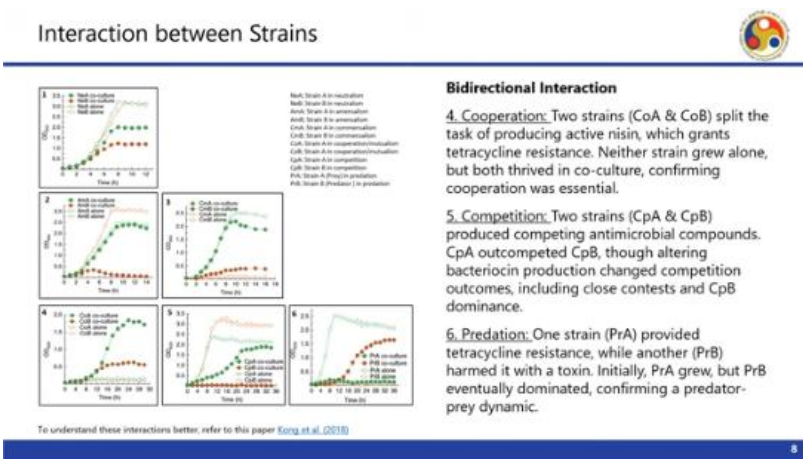
Here you can see CmA grows alone in tetracycline media due to built-in resistance, while CmB cannot. However, in co-culture, CmA's nisin production activates CmB's resistance, enabling its growth. Both strains compete for nutrients, reducing CmA's growth in co-culture. Then we have the bidirectional interactions. In the case of four, you can see two strains, CoA and CoB, split the task of producing active nisin, which grants tetracycline resistance.



Neither strain grew alone, but both thrive in co-culture, confirming cooperation was essential. Then in the fifth case, there is competition. The two strains, competitive strains CpA and CpB, produce nisin. Competing antimicrobial compounds, CpA outcompeted

CpB. So, altering bacteria's production changed competition outcomes, including close contest and CpB dominance.

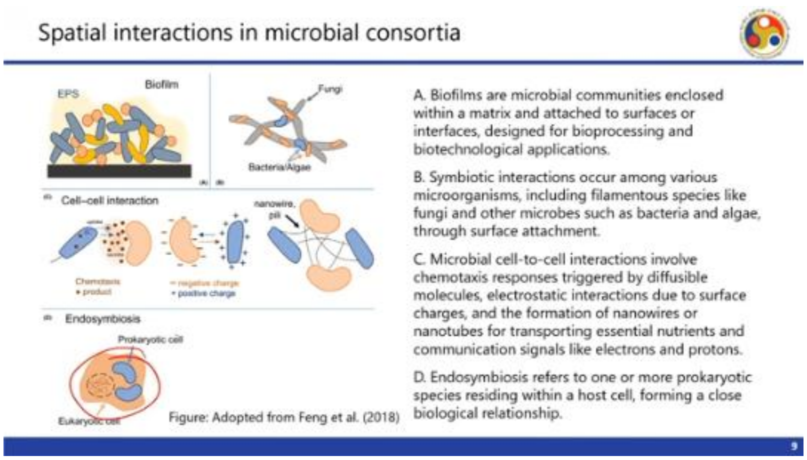
In the case of six, we have predation. One strain, PrA, provided tetracycline resistance, while another, PrB, harmed it with a toxin. Initially, PrA grew, but PrB eventually dominated, confirming a predator-prey dynamic. Let us now look into the spatial interactions in microbial consortia. So, this is a biofilm with many bacterial species over here, and you can see the exopolysaccharide matrix over there. In the case of B, you can see these are fungi to which some bacteria are absorbed or attached. So, biofilms are microbial communities that are enclosed within a matrix and attached to surfaces or interfaces designed for bioprocessing and biotechnological applications.



In symbiotic relationships, interactions occur among various microorganisms, including filamentous species like fungi and other microbes, bacteria, and sometimes algae, through surface attachment. Now, in another example of spatial interactions, we have cell-cell interactions where there is a chemotaxis product. So, this involves chemotaxis responses together with diffusible molecules that are secreted by one, and then electrostatic interactions, as you can see here, due to surface charges and the formation of nanowires or nanotubes for transporting essential nutrients and communication signals like electrons and protons. So here, bacterial cells of different strains or different species are trying to communicate with one another through either electrostatic interactions, sending molecules, or establishing physical contact via nanowires.

Then we have endosymbiosis. Here, one or more prokaryotic species reside within a host cell, forming a close biological relationship. So this is a large eukaryotic cell that is now hosting a prokaryotic cell, and they exist in a mutually beneficial symbiotic relationship.

So this we call endosymbiosis because one species is inside the other. So, what are the advantages of microbial consortia?

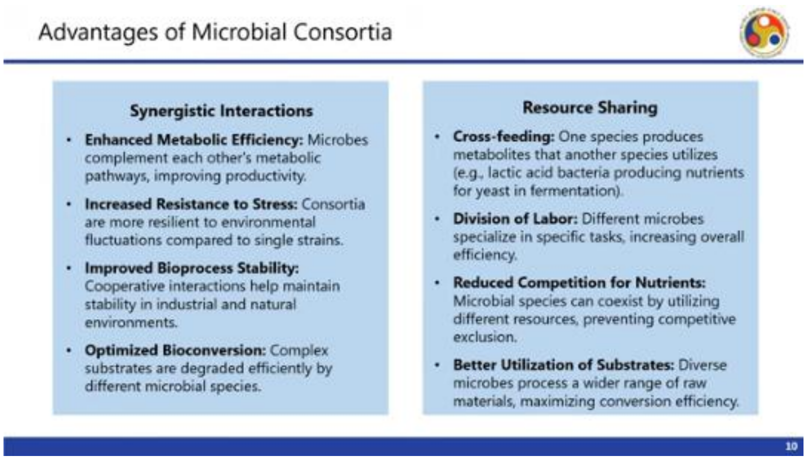


They provide synergistic interactions, due to which enhanced metabolic efficiency is achieved. Microbes complement each other's metabolic pathways, improving productivity. There is increased resistance to stress. Consortia are more resilient to environmental fluctuations compared to single strains. There is improved bioprocess stability.

Cooperative interactions help maintain stability in industrial and natural environments. There is an optimized bioconversion opportunity. Complex substrates are degraded efficiently by different microbial species. The second advantage is resource sharing. There is cross-feeding.

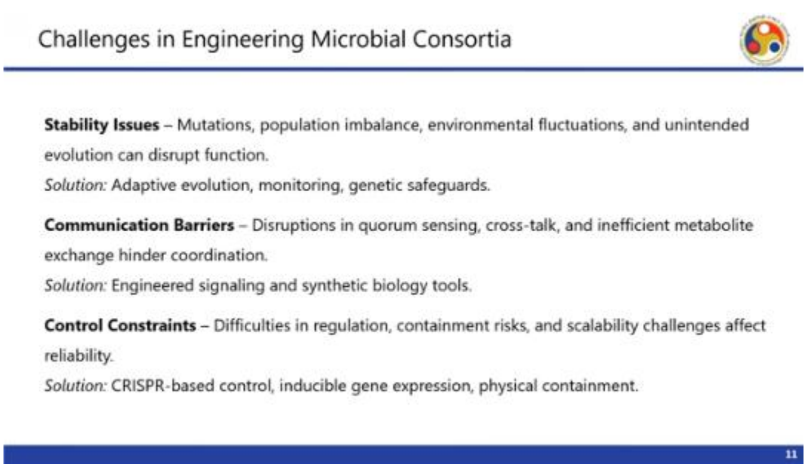
One species produces metabolites that another species utilizes. For example, lactic acid bacteria produce nutrients for yeast in fermentation. There is a division of labor. Different microbes specialize in specific tasks, increasing overall efficiency. There is reduced competition for nutrients.

Microbial species can coexist by utilizing different resources, preventing competitive exclusion. There is better utilization of substrates. Diverse microbes process a wider range of raw materials, maximizing conversion efficiency. What are the challenges in engineering microbial consortia? The first and foremost issue is the stability issue. Mutations, population imbalance, environmental fluctuations, and unintended evolution can disrupt function.



The solution for this is adaptive evolution, monitoring, and genetic safeguards. Then there are the communication barriers: disruptions in quorum sensing, crosstalk, and inefficient metabolite exchange hinder coordination. The solution to these communication barrier problems is engineered signaling and synthetic biology tools. Then there is the challenge of control constraints. There are difficulties in regulation, containment risks, and scalability challenges, which affect reliability.

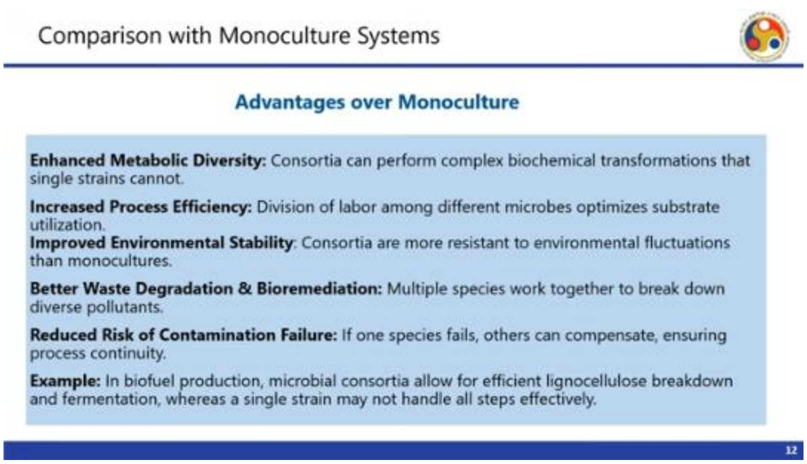
The solution for these can be found in CRISPR-based control, inducible gene expression, and physical containment. Now let us compare microbial consortia with monoculture systems. They have advantages over monocultures. Here, there is enhanced metabolic diversity. Consortia can perform complex biochemical transformations that single strains cannot achieve.



There is increased process efficiency. Division of labor among the different microbes optimizes substrate utilization. There is improved environmental stability. Consortia are more resistant to environmental fluctuations than monocultures. There is better waste degradation and bioremediation.

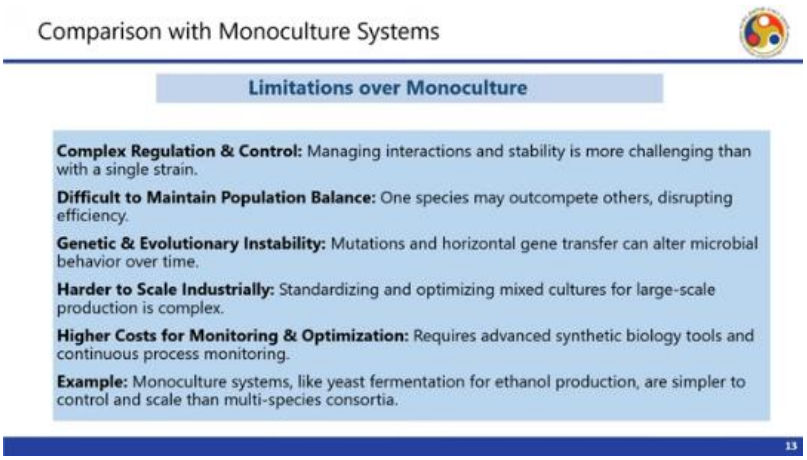
Multiple species work together to break down diverse pollutants. There is a reduced risk of contamination failure. If one species fails, others can compensate, ensuring process continuity. But in the case of a single culture, we do not have this luxury. So, let's have some examples.

For instance, in biofuel production, microbial consortia allow for efficient lignocellulose breakdown and fermentation, whereas a single strain may not handle all steps effectively. What are the limitations of monoculture? When we see that certain microbial consortia may be difficult from the point of view of complex regulation and control, managing interactions and stability is more challenging than with a single strain. Then, it is difficult to maintain population balance. One species may outcompete others, disrupting efficiency and thereby transforming it into a single strain after a few rounds.

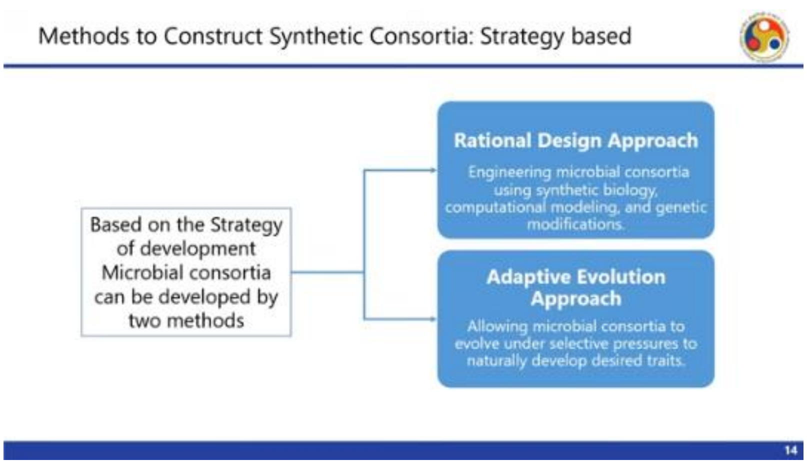


Then there is genetic and evolutionary instability. Mutations and horizontal gene transfer can alter microbial behavior over time. It is also harder to scale them industrially. Standardizing and optimizing mixed cultures for large-scale production is a very complex process. It also suffers from higher costs involving monitoring and optimization.

So, it requires advanced synthetic biology tools and continuous process monitoring. Some examples, for instance, are molecules or systems like yeast fermentation for ethanol production, which are simpler to control and scale than multi-species consortia. What are the various methods in our disposal to construct synthetic consortia? The number one is the strategy-based. Based on the strategy of development, microbial consortia can be developed by two methods.

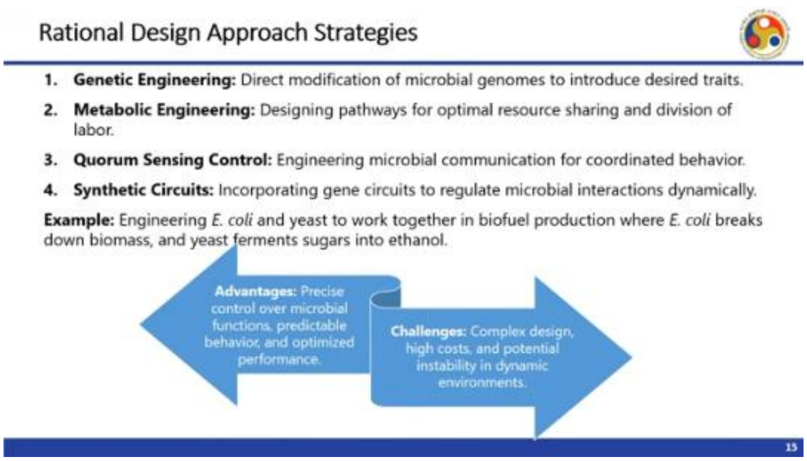


The first method is the rational design approach. Here, engineering microbial consortia is done using synthetic biology, computational modeling, and genetic modification. The other is the adaptive evolution approach, where we allow microbial consortia to evolve under selective pressures to naturally develop desired traits. So, in a rational design approach strategy, we employ genetic engineering, which is a direct modification of microbial genomes to introduce desired traits. We also go for metabolic engineering, whereby we design pathways for optimal resource sharing and division of labor.



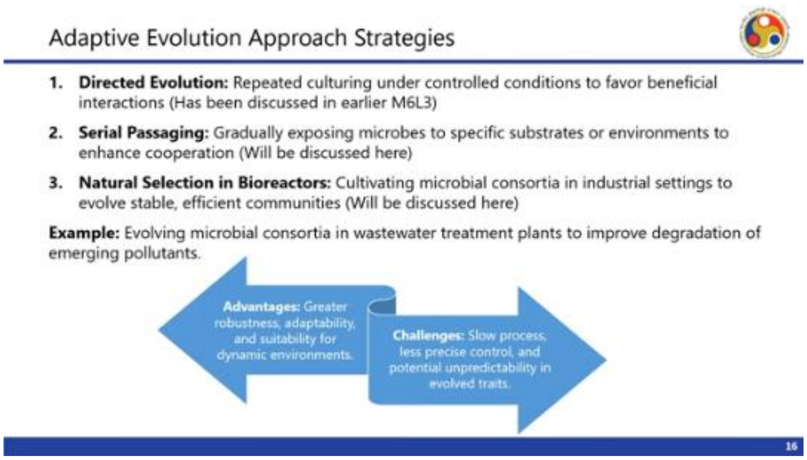
Then we go for quorum sensing control. Here, we engineer microbial communication for coordinated behavior. Then we also use synthetic circuits by incorporating gene circuits to regulate microbial interactions dynamically. For example, engineering Escherichia coli and yeast to work together in biofuel production, where E. coli breaks down biomass and yeast ferments sugars into ethanol. So, what are the advantages of rational design approach strategies?

It is precise, provides control over microbial functions, predictable behavior, and optimized performance. However, the challenges associated with rational design are complex design strategies, high costs, and potential instability in dynamic environments. Let's discuss the adaptive evolution approach strategy, where we use directed evolution. Here, repeated culturing under controlled conditions favors beneficial interactions. So, this was already discussed in the earlier lecture.



Then we again go for serial processing, where we gradually expose microbes to specific substrates or environments to enhance cooperation. Then we go for natural selection in bioreactors, cultivating microbial consortia in industrial settings to evolve stable, efficient communities. So, some examples of adaptive evolution approach strategies include evolving microbial consortia in wastewater treatment plants to improve degradation of emerging pollutants. So, what are the advantages of the adaptive evolution approach strategy? It offers greater robustness, adaptability, and suitability for dynamic environments.

However, it also suffers from various challenges like being a very slow process, offering less precise control and potential unpredictability in evolved strains. Let's compare some of the adaptive evolution strategies like directed evolution, serial passaging, and natural selection in bioreactors. So, when it comes to selection mechanisms, directed evolution is a human-directed screening or selection for specific traits. In serial passaging, adaptation occurs through periodic bottlenecks and environmental shifts. In natural selection in bioreactors, environmental conditions drive natural selection.



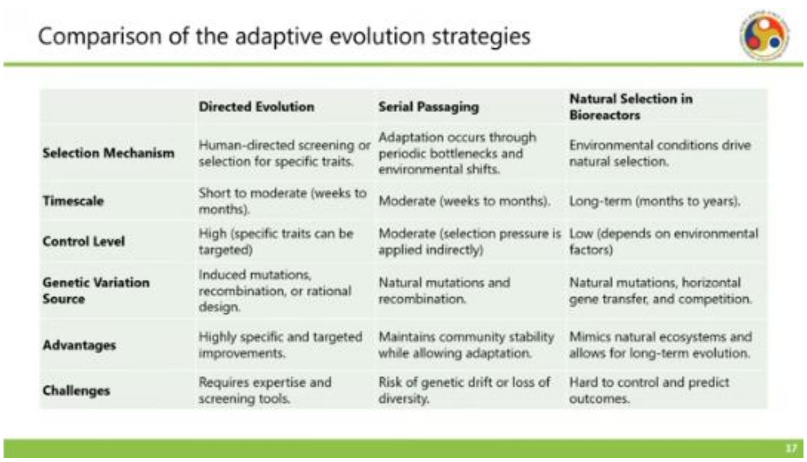
From the point of time scale, directed evolution is short to moderate, taking weeks to months. Serial passaging is moderate, taking weeks to months. Natural selection in bioreactors is actually a long-term process. It may take months to years. Then, from the point of control view,

In directed evolution, the control level is high. Specific traits can be targeted. In serial passaging, it is moderate. Selection pressure is applied indirectly. In the case of natural selection, the control level is very low.

It depends on environmental factors. The source of genetic variation in the case of directed evolution is induced mutations, recombinations, or rational designs. In the case of serial passaging and natural selection in bioreactors, it is natural mutations and recombinations. In natural selection in bioreactors, we can also have horizontal gene transfer as well as competition.

So, what are the advantages of directed evolution? It is highly specific, and targeted improvements can take place there. In serial passaging, community stability is maintained while allowing adaptation. Natural selection in bioreactors mimics a natural ecosystem and also enables long-term evolution. There are various challenges. For example, directed evolution requires experience and screening tools. In serial passaging, there is a risk of genetic drift or loss of diversity.

In the case of natural selection in bioreactors, it is hard to control and predict outcomes. Now, let's look into the methods to construct synthetic consortia source-based. These can be developed by two methods. One is the top-down approach, where engineering microbial consortia using synthetic biology, computational modeling, and genetic modifications takes place. Then there is the bottom-up approach, where we allow microbial consortia to evolve under selective processes to naturally develop desired traits.



Comparison of the adaptive evolution strategies

	Directed Evolution	Serial Passaging	Natural Selection in Bioreactors
Selection Mechanism	Human-directed screening or selection for specific traits.	Adaptation occurs through periodic bottlenecks and environmental shifts.	Environmental conditions drive natural selection.
Timescale	Short to moderate (weeks to months).	Moderate (weeks to months).	Long-term (months to years).
Control Level	High (specific traits can be targeted)	Moderate (selection pressure is applied indirectly)	Low (depends on environmental factors)
Genetic Variation Source	Induced mutations, recombination, or rational design.	Natural mutations and recombination.	Natural mutations, horizontal gene transfer, and competition.
Advantages	Highly specific and targeted improvements.	Maintains community stability while allowing adaptation.	Mimics natural ecosystems and allows for long-term evolution.
Challenges	Requires expertise and screening tools.	Risk of genetic drift or loss of diversity.	Hard to control and predict outcomes.

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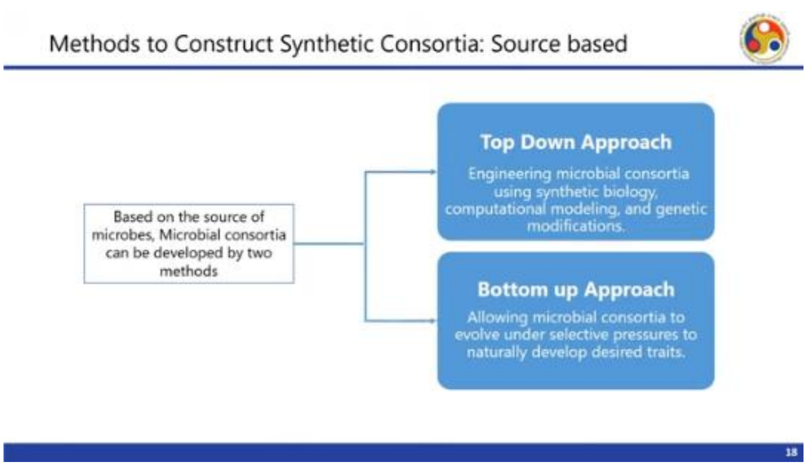
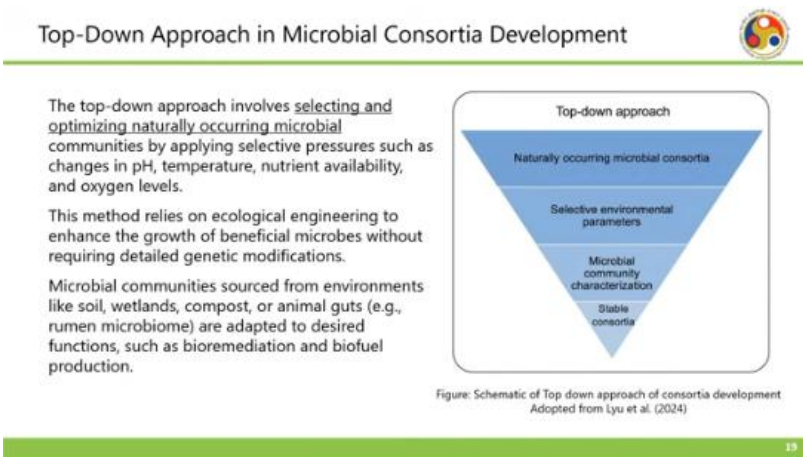
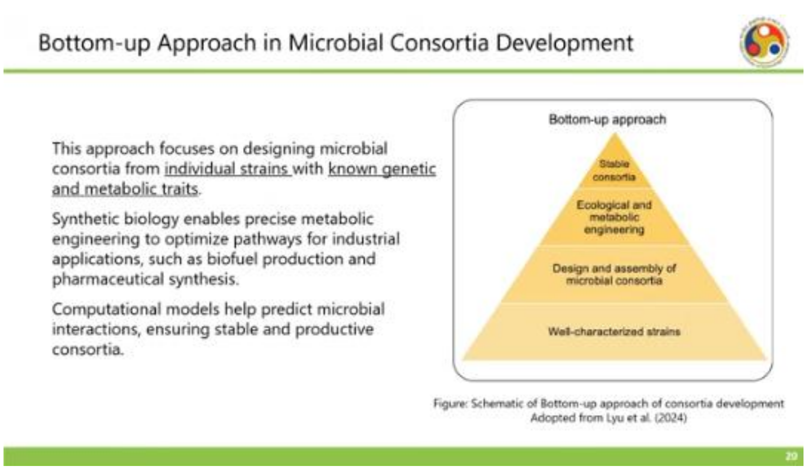


So, let's discuss the top-down approach in microbial consortia development first. The top-down approach involves selecting and optimizing naturally occurring microbial communities by applying selective pressures, such as changes in pH, temperature, nutrient availability, and oxygen levels. So, as you can see, it's a top-down approach that starts at the top. Here, naturally occurring microbial consortia are first taken, then we apply selective environmental parameters, followed by the characterization of the microbial community, and we try to achieve stable consortia for further work. This method relies on ecological engineering to enhance the growth of beneficial microbes without requiring detailed genetic modifications.

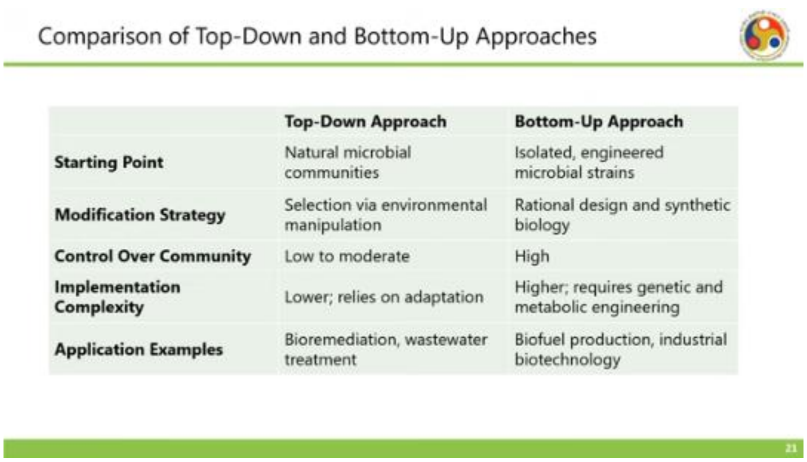
Microbial communities sourced from environments such as soil, wetlands, compost, or animal guts (e.g., rumen microbiome) are adapted to desired functions, such as bioremediation and biofuel production. In the case of the bottom-up approach in microbial consortia development, we focus on designing microbial consortia from individual strains with known genetic and metabolic traits. So, we start from the bottom, where we take various well-characterized strains and design and assemble these into a consortia, involving ecological and metabolic engineering to obtain stable microbial consortia. Synthetic biology enables precise metabolic engineering to optimize pathways for industrial applications, such as biofuel production and pharmaceutical synthesis.



Computational models help predict microbial interactions, ensuring stable and productive consortia. Now, let us compare these two approaches to see which one has what kind of advantages and disadvantages. From the point of view of the starting point, the top-down approach takes natural microbial communities, while the bottom-up approach takes isolated, engineered microbial strains. From the point of modification, in the top-down approach, selection occurs via environmental manipulation. In the bottom-up approach, it is rational design and synthetic biology.



From the point of view of control over the community, in the top-down approach, it is low to moderate. In the bottom-up approach, it is very, very high. From the point of view of implementation complexity, the top-down approach has less complexity and relies on adaptation. The bottom-up approach has higher complexity and requires genetic and metabolic engineering. From examples, we can see top-down bioremediation in wastewater treatment and bottom-up biofuel production in industrial biotechnology.

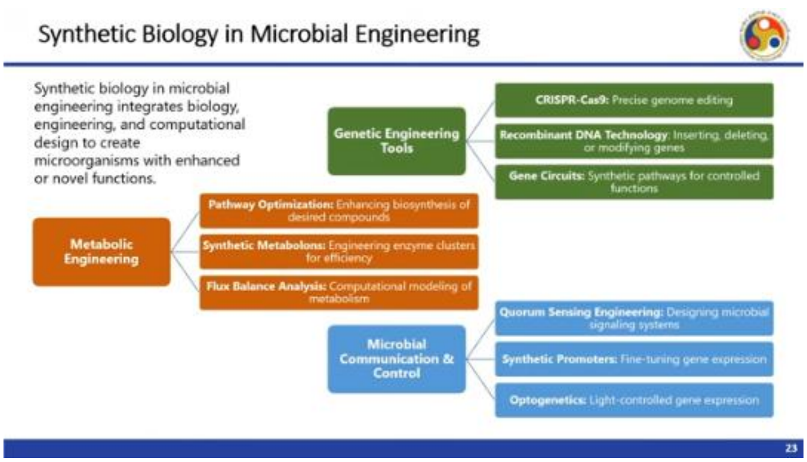

Comparison of Top-Down and Bottom-Up Approaches

	Top-Down Approach	Bottom-Up Approach
Starting Point	Natural microbial communities	Isolated, engineered microbial strains
Modification Strategy	Selection via environmental manipulation	Rational design and synthetic biology
Control Over Community	Low to moderate	High
Implementation Complexity	Lower; relies on adaptation	Higher; requires genetic and metabolic engineering
Application Examples	Bioremediation, wastewater treatment	Biofuel production, industrial biotechnology

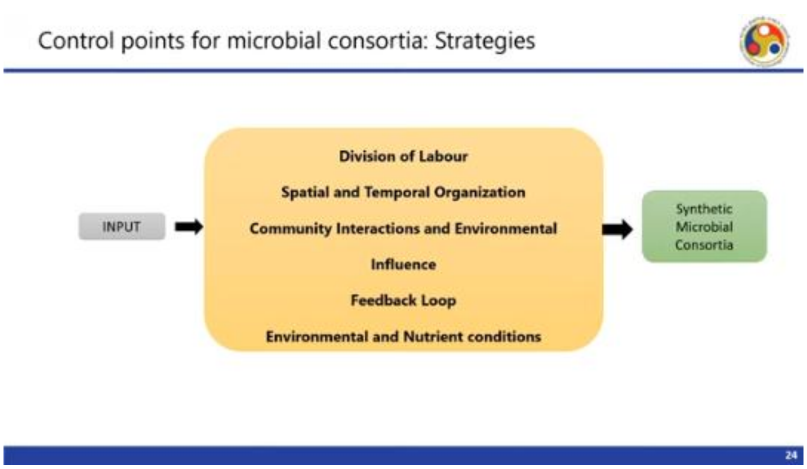
Let's now move to section number two, where we will discuss synthetic biology in microbial engineering. Control points for microbial consortia strategies, division of labor, spatial and temporal organization, community interactions and environmental influences, interactions through quorum sensing, enhanced interactions, feedback loops, and environmental and nutrient conditions. Then we will also discuss gene circuit design for microbial communication, a modular approach to engineering microbial consortia, metabolic engineering for efficient bioprocessing, and computational modeling of microbial consortia. So, let's start with synthetic biology in microbial engineering.

Synthetic biology in microbial engineering integrates biology, engineering, and computational design to create microorganisms with enhanced or novel functions. We use various gene editing and genetic engineering tools. For example, recombinant DNA technology, whereby we insert, delete, or modify genes. Then we use genome editing tools like CRISPR-Cas9 for precise manipulation. We also use synthetic circuits with pathways for control functions.

Then we use tools of metabolic engineering, for example, pathway optimization and enhanced biosynthesis of desired compounds. Then we go for synthetic metabolomes, which involve engineering enzyme clusters for efficiency. Then we go for flux balance analysis, where computational modeling of metabolism is done. Then we use microbial communication and control. Here, concepts like quorum sensing engineering, designing microbial signaling systems, synthetic promoters for fine-tuning gene expression, and optogenetics for light-controlled gene expression are adopted or used. These provide an overall overview of the tools and techniques in synthetic engineering. We are providing only a few examples; there are many more, which we will discuss from time to time.

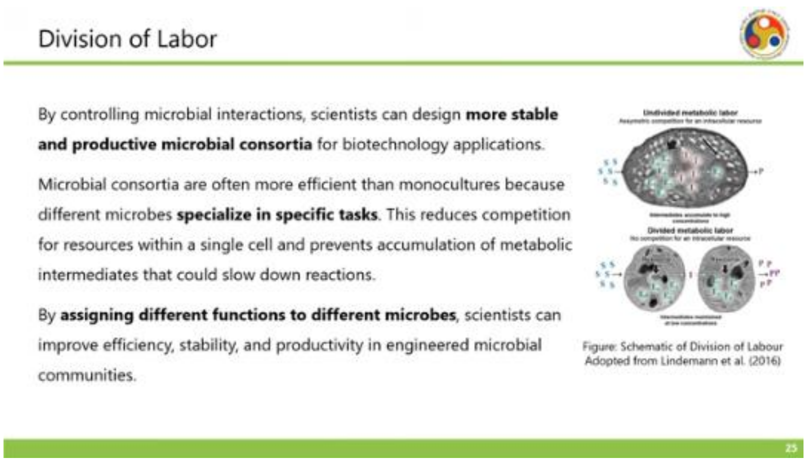


So, what are the control points for microbial consortia and the strategies? There is an input that helps in the division of labor, and then there is spatial and temporal organization. There is community interaction and environmental influence, and then there is a feedback loop involving environmental and nutrient conditions. This leads to the synthetic microbial consortia. The most important thing is the division of labor.



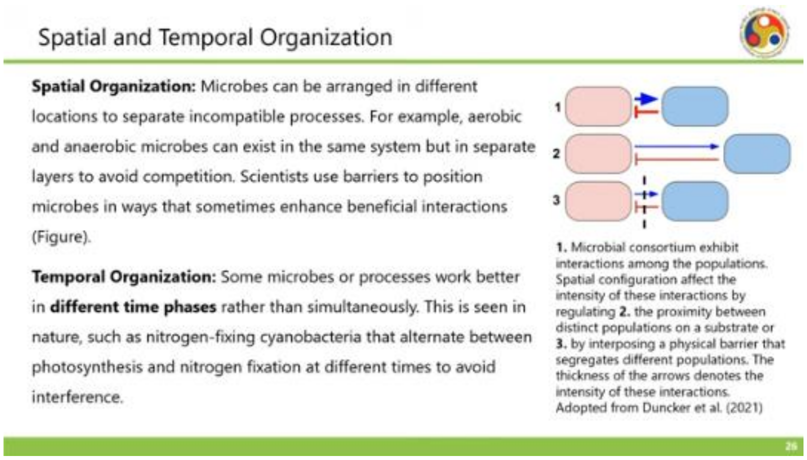
By controlling microbial interactions, we can design more stable and productive microbial consortia for biotechnology applications. Microbial consortia are often more efficient than monocultures because different microbes specialize in specific tasks. This reduces competition for resources within a single cell and prevents the accumulation of metabolic intermediates that could slow down reactions. By assigning different functions to different microbes, we can improve efficiency, stability, and productivity in engineered microbial communities. Now, here you can see in the first case of undivided metabolic labor, there is symmetric competition for intracellular resources, and these intermediates accumulate to high concentrations.

Then there is a divided metabolic labour, there is no competition for an intracellular resource, so intermediates maintained at low concentration. Then comes the spatial and temporal organization. Microbes can be arranged in different locations to separate incompatible processes. For example, aerobic and anaerobic microbes can exist in the same system but in separate layers to avoid competition. Scientists use barriers to position microbes in ways that sometimes enhance beneficial interactions.

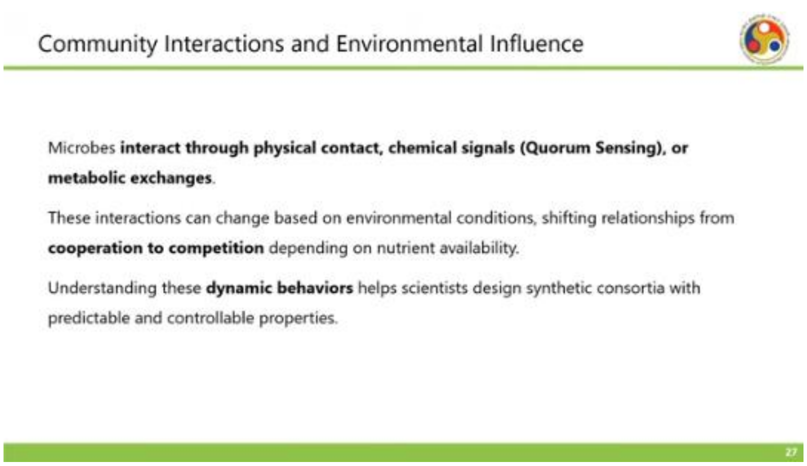


So, we can see this in the microbial consortium. Number one, exhibiting interactions among the populations, spatial configurations affect the intensity of these interactions by regulation. Then in number two, we can see the proximity between distinct populations on a substrate or in number three, by interposing a physical barrier as you can see over here, here, that segregates different populations. The thickness of the arrows denotes the intensity of these interactions.

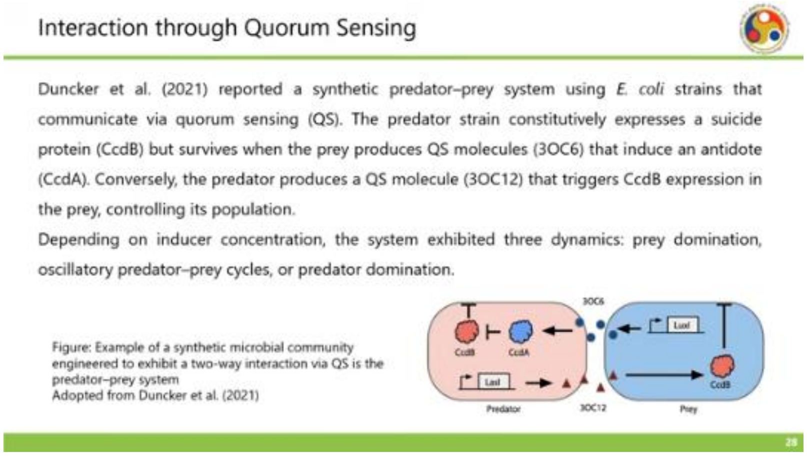
So, in temporal organizations, some microbes or processes work better in different time phases rather than being in the same time, same space. This is seen in nature such as nitrogen-fixing cyanobacteria that alternate between photosynthesis and nitrogen fixation at different times to avoid interference. So, microbes interact through physical contact, chemical signals quorum sensing, or metabolic exchanges. So, as a community, they have a lot of interactions and also influenced by environment.



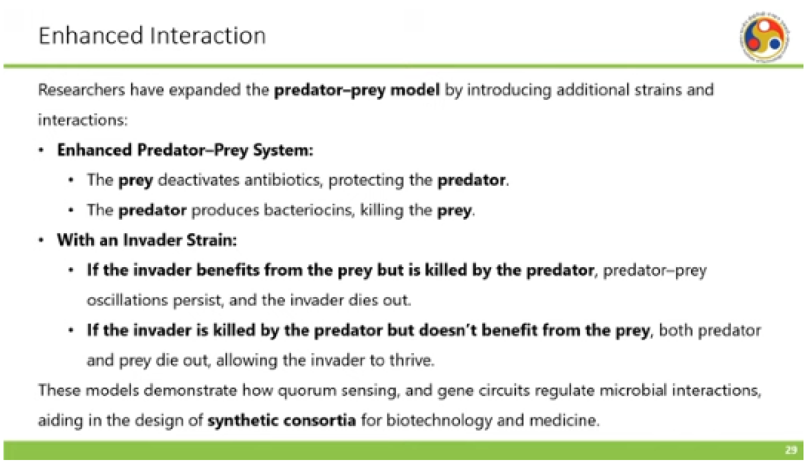
These interactions can change based on environmental conditions, shifting relationships from cooperation to competition depending on nutrient availability. Understanding these dynamic behaviors helps scientists design synthetic consortia with predictable and controllable properties. What are the interactions through quorum sensing, and how does it take place? Dunker in 2021 reported a synthetic predator-prey system using E. coli strains that communicate via quorum sensing. The predator strain constitutively expresses a suicide protein, CcdB, but survives when the prey produces a QS molecule, 3OC6, that induces an antidote, CcdA, conversely.



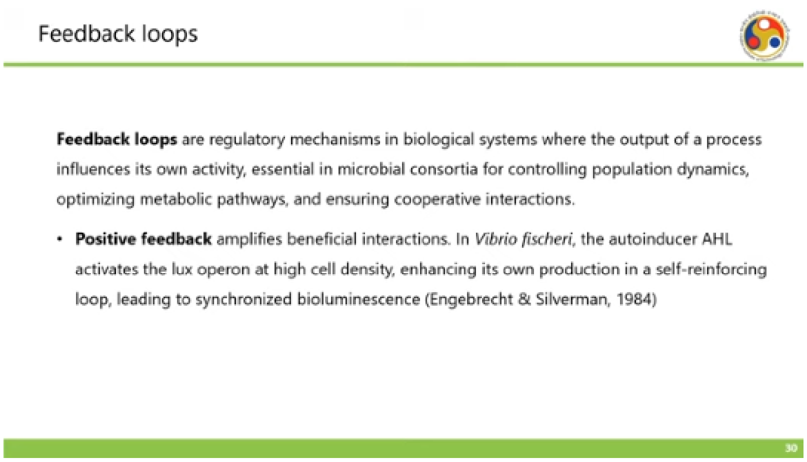
The predator produces a QS molecule, 3OC12, that triggers CcdB expression in the prey, controlling its population. Depending on inducer concentration, the system exhibited three dynamics: prey domination, oscillatory predator-prey cycles, or predator domination. So here in this figure, you can see an example of a synthetic microbial community engineered to exhibit a two-way interaction via QS. The predator-prey CC system CcdB is here, which is a suicide protein, and then you have this antidote, CcdA. So, as already discussed, depending on the inducer concentration, This whole system will exhibit three different situations: prey domination, oscillatory predator-prey cycles, or predator domination. So let's discuss the enhanced interactions. So here, researchers have expanded this predator-prey model by introducing additional strains and interactions. In the enhanced predator-prey system, the prey deactivates antibiotics, thereby protecting the predator. The predator produces bactericides, killing the prey.



With an invader strain, if the invader benefits from the prey but is killed by the predator, predator-prey oscillations persist, and the invader dies out. If the invader is killed by the predator but doesn't benefit from the prey, both predator and prey will die, allowing the invader to thrive. These models demonstrate how quorum sensing and gene circuits regulate microbial interactions, aiding in the design of synthetic consortia for biotechnology and medicine. Then there is the role of feedback loops, which are regulatory mechanisms in biological systems where the output of a process influences its own activity. Essentially, in microbial consortia, they control population dynamics,

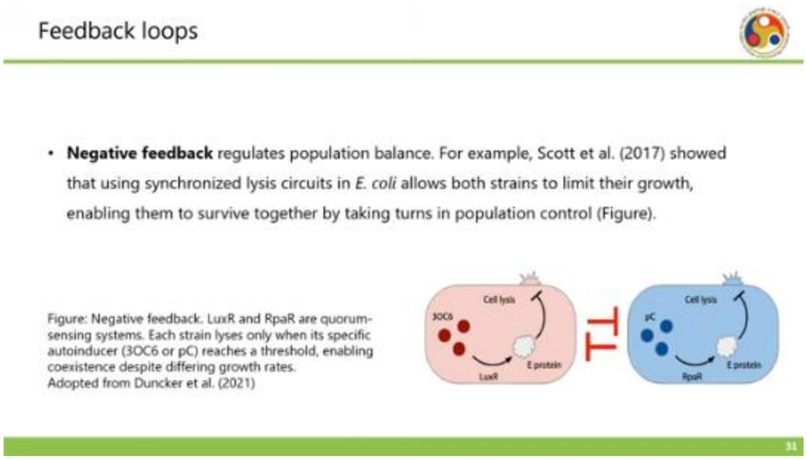


optimize metabolic pathways, and ensure cooperative interactions. So, we have positive feedback, which amplifies beneficial interactions. In Vibrio fischeri, the autoinducer AHL activates the lux operon at high cell density, enhancing its own production in a self-reinforcing loop, leading to synchronized bioluminescence. Then there is negative feedback, which regulates population balance.

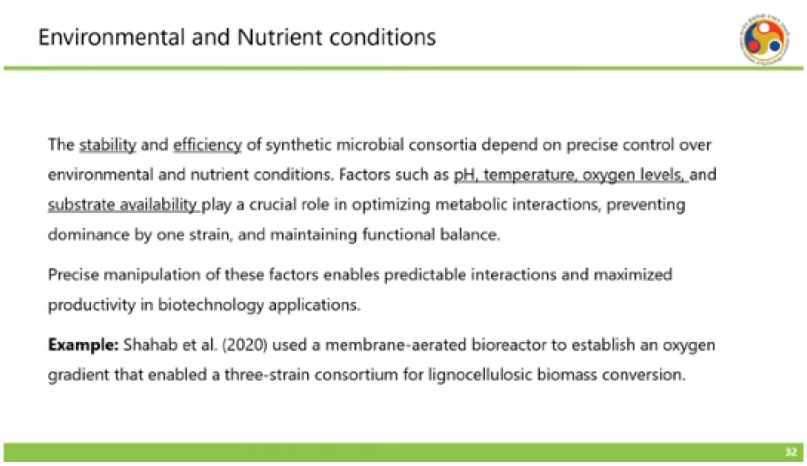


For example, Scott et al. showed that using synchronized lysis circuits in E. coli allows both strains to limit their growth, enabling them to survive together by taking turns in population control. So, here we can see negative feedback. Lux-R and Rpa-R are quorum-sensing systems. Each strain lyses only when its specific autoinducer, 3OC6 or PC, reaches a threshold, enabling coexistence.

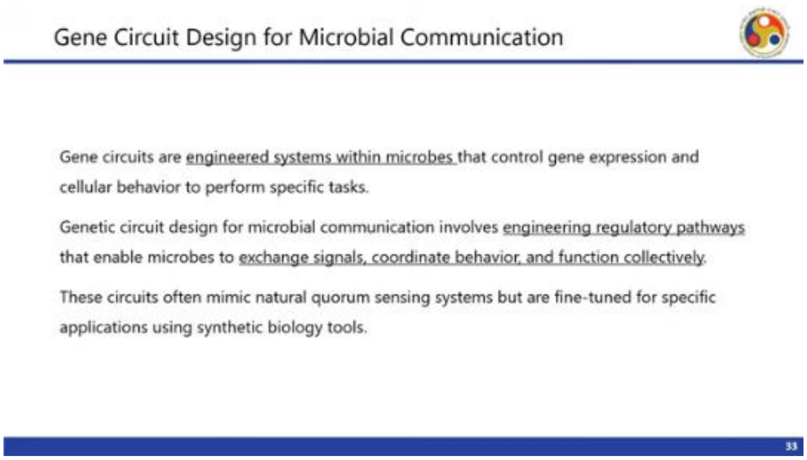
Despite differing growth rates. This figure we have adopted from the work of Dankar et al. Let's discuss the environmental and nutrient conditions. The stability and efficiency of synthetic microbial consortia depend on precise control over environmental and nutrient conditions. Factors such as pH, temperature, oxygen levels, and substrate availability play a crucial role in optimizing metabolic interactions, preventing dominance by one strain, and maintaining functional balance.



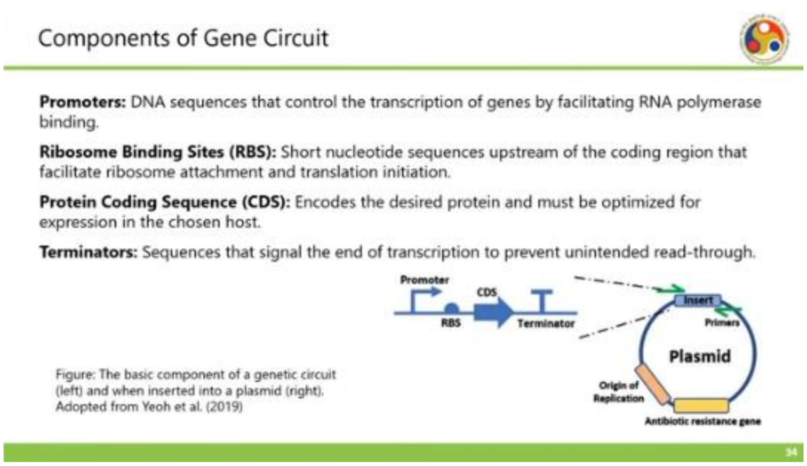
Precise manipulation of these factors enables predictable interactions and maximizes productivity in biotechnology applications. For example, in this case by Shahab, they used a membrane-aerated bioreactor to establish an oxygen gradient and enable the three-strain consortium for lignocellulosic biomass conversion. Let us now look into the gene circuit design for microbial communication. These gene circuits are engineered systems within microbes that control gene expression and cellular behavior to perform specific tasks. Genetic circuit design for microbial communication involves engineering regulatory pathways that enable microbes to exchange signals, coordinate behavior, and function collectively.



These circuits often mimic natural quorum sensing systems but are fine-tuned for specific applications using synthetic biology tools. What are the components of a gene circuit? Promoters come first: DNA sequences that control the transcription of genes by facilitating RNA polymerase binding. Then we have the ribosome binding sites. These are short nucleotide sequences upstream of the coding region that facilitate ribosome attachment and the initiation of translation.

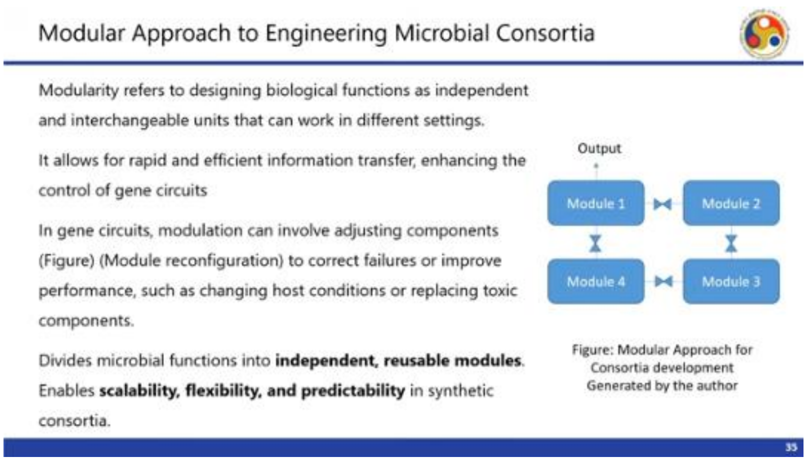


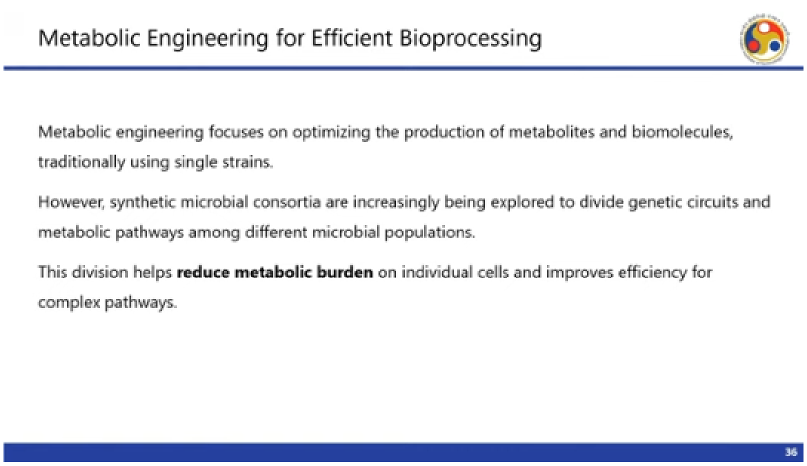
Then there is the protein coding sequence or CDS. These encodes the desired protein and must be optimized for expression in the chosen host then the terminators, which are the sequences that signal the end of transcription to prevent unintended read-through. So this figure, you can see the basic components of a genetic circuit, the promoter ribosome bonding site, then protein coding sequence and the terminator, which are together constitutes the insert and this is cloned into a vector, which may be a plasmid. What is the modular approach to engineering microbial consortia?



Modularity refers to designing biological functions as independent and interchangeable units that can work in different settings. It allows for rapid and efficient information transfer enhancing the control of circuits. In gene circuits, modulation can involve adjusting components So, here you see there are four modules, one, two, three and four. The module reconfiguration is important to correct failures or improve performance, such as changing host conditions or replacing toxic components.

It divides the microbial functions into independent, reusable modules, enables scalability, flexibility and predictability in synthetic consoles here. Now let us discuss about the metabolic engineering for efficient bioprocessing. Metabolic engineering focuses on optimizing the production of metabolites and biomolecules traditionally using single strains. However, synthetic microbial consortia are increasingly being explored to divide genetic circuits and metabolic pathways among different microbial populations. This division helps reduce metabolic burden on individual cells and improves efficiency for complex pathways.

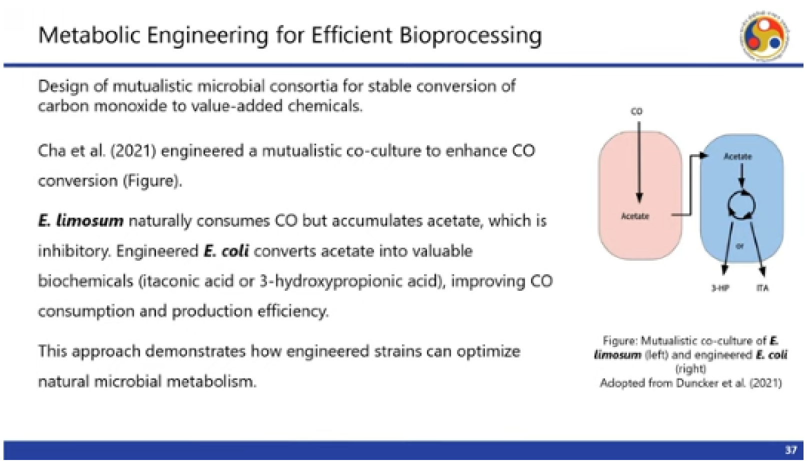




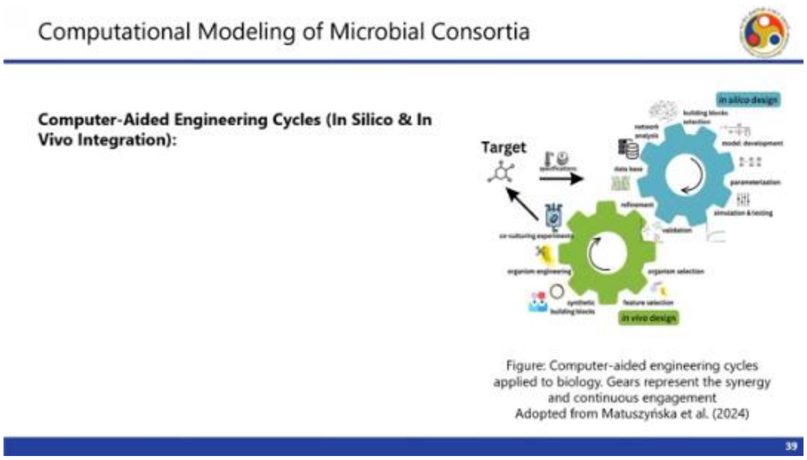
So, let us now look into some examples where Cha et al. designed a mutualistic microbial consortia for stable conversion of carbon monoxide to produce value-added chemicals. So, in this picture, you can see the carbon monoxide. Going through the cell and then converting it to acetate, which is then transferred to another cell in the consortia, which will convert it into different value-added products. So, this Escherichia limosum naturally consumes carbon monoxide but accumulates acetate, which is inhibitory. So, engineered Escherichia coli converts this acetate into valuable biochemicals like itaconic acid or 3-

hydroxypropionic acid, improving carbon monoxide consumption and production efficiency.

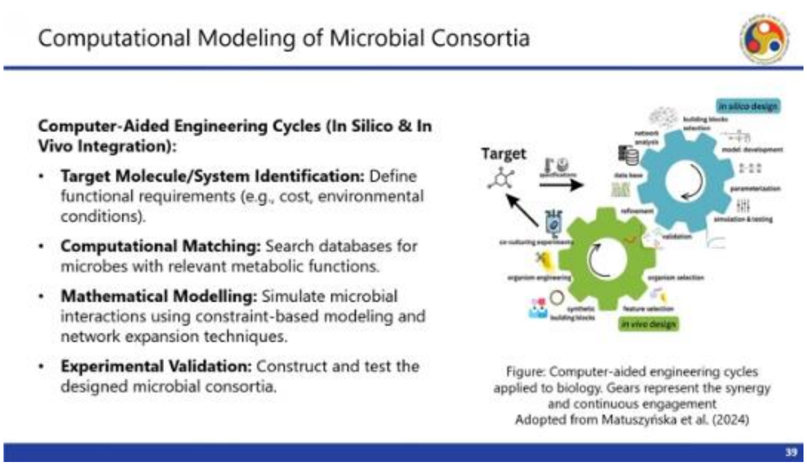
This approach demonstrates how engineered strains can optimize natural microbial metabolism. Let us now discuss computational modeling of microbial consortia. This is revolutionizing the design of synthetic ecosystems, bioprocess engineering, and environmental biotechnology. By shifting from species-centered to function-centered modeling, researchers can design robust, efficient microbial communities for applications in bioremediation, bioenergy, agriculture, and medicine. So, this organism-free modular approach includes microbial species, which are viewed as chassis containing essential metabolic pathways, and they play functional roles like metabolite production, nutrient provision, and antimicrobial defense. These are treated as independent modules that can be computationally optimized.



Then, databases of these functional modules could resemble biobricks or the registry of standard biological parts for synthetic biology. Computer-aided engineering cycles in silico and in vitro integration. So here, the gears represent the synergy and continuous engagement. Target molecule and system identification are important. We define the functional requirements, for example, what is the cost and environmental conditions.



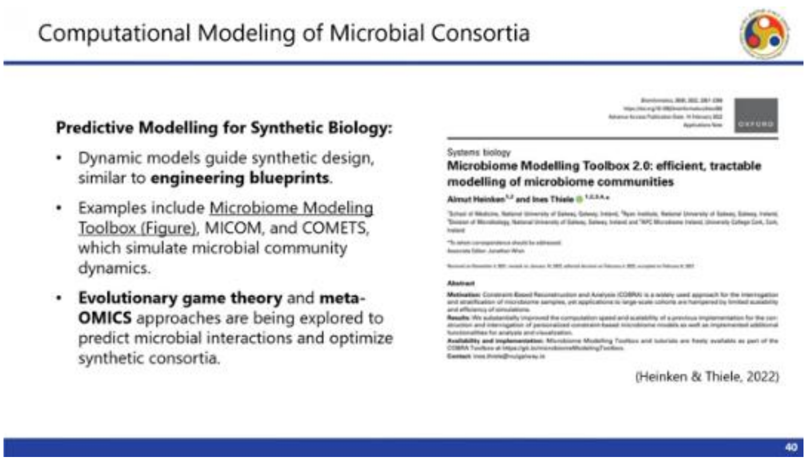
Then we go for computational matching, searching the database for microbes with relevant metabolic functions. Then we also undertake mathematical modeling by simulating microbial interactions using constraint-based modeling and network expansion techniques. And finally, we validate this model, construct, and test the designed microbial consortia. So there are various steps involved in it and also various resources required for carrying out this computational modeling for microbial consortia. But this actually helps us in developing very efficient consortia.



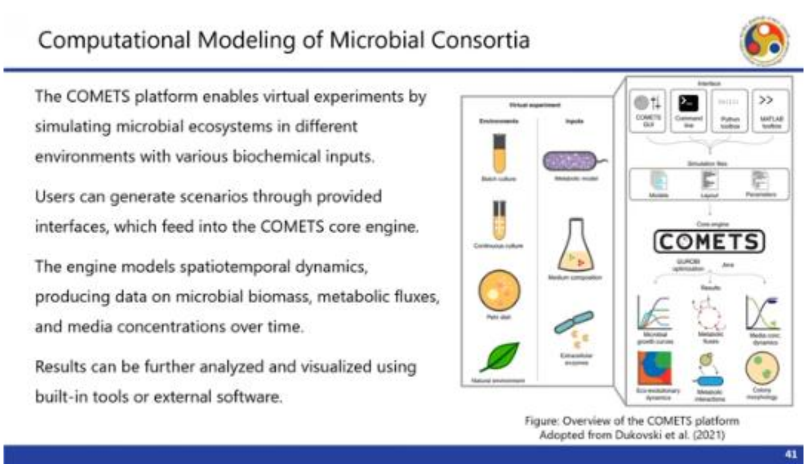
So, there is predictive modeling for synthetic biology. So, here you have the Microbiome Modeling Toolbox 2.0, which enables efficient, tractable modeling of microbiome communities. Dynamic models guide synthetic design, similar to engineering blueprints. Some examples include microbiome modeling, as suggested by the systems biology paper. Then MICOM and COMETS, which simulate microbial community dynamics.

Then there is evolutionary game theory and the meta-omics approach, which are being explored to predict microbial interactions and optimize synthetic consortia. So, the

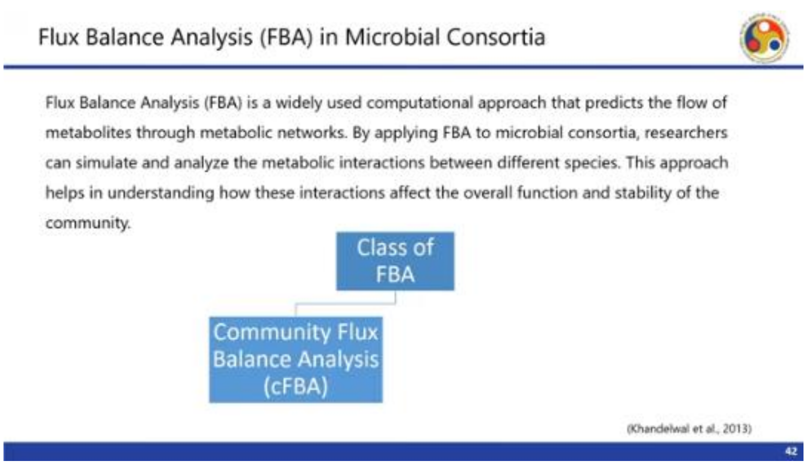
COMETS platform in this figure enables virtual experiments by simulating microbial ecosystems in different environments with various biochemical inputs. So, here you have these environments and these inputs, then metabolic models. Then we have batch cultures, continuous culture, medium composition, Petri dish, natural environment, and extracellular environment. Users can generate scenarios through provided interfaces, which feed into the COMETS core engine, as you can see over here.

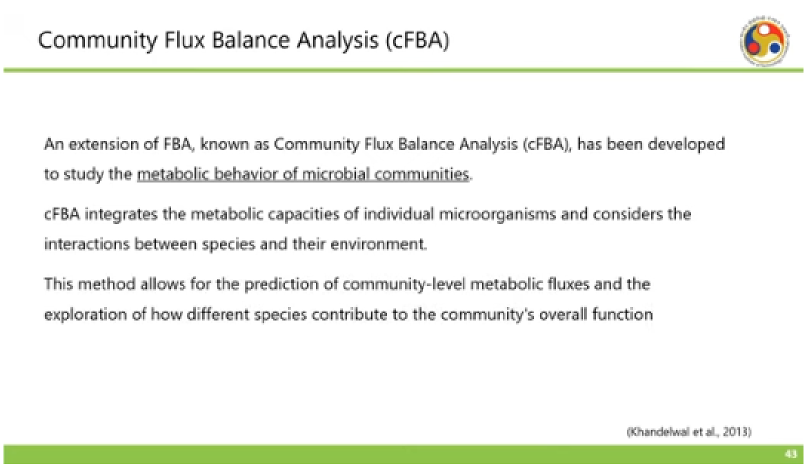


This engine models spatio-temporal dynamics, producing data on microbial biomass, metabolic fluxes, and media concentrations over time. The results can be further analyzed and visualized using built-in tools or external software. Another important aspect is flux balance analysis in microbial consortia. This FBA is a widely used computational approach that predicts the flow of metabolites through metabolic networks. By applying FBA to microbial consortia, researchers can simulate and analyze the metabolic interactions between different species.

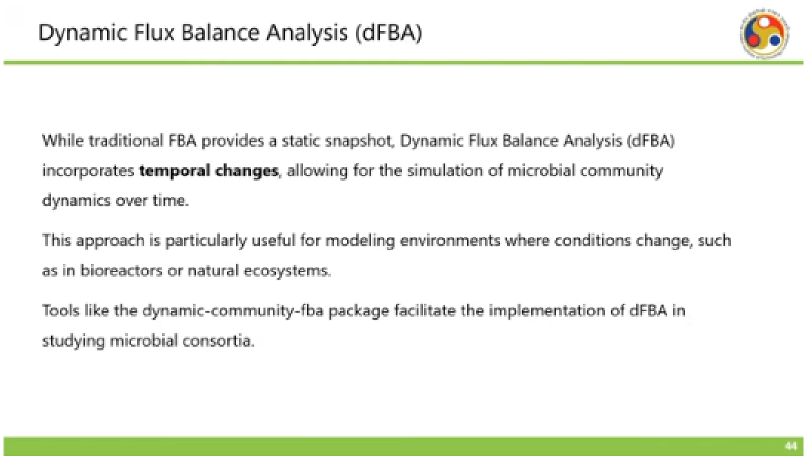


This approach helps in understanding how these interactions affect the overall functions and stability of the community. So, there are different classes of FBA: community flux balance analysis (cFBA) and dynamic flux balance analysis (dFBA). Community flux balance analysis is an extension of FBA, known as community flux balance analysis, developed to study the metabolic behavior of microbial communities. It integrates the metabolic capacities of individual microorganisms and considers the interactions between species and their environment. This method allows for the prediction of community-level metabolic fluxes and exploration of how different species contribute to the community's overall function.



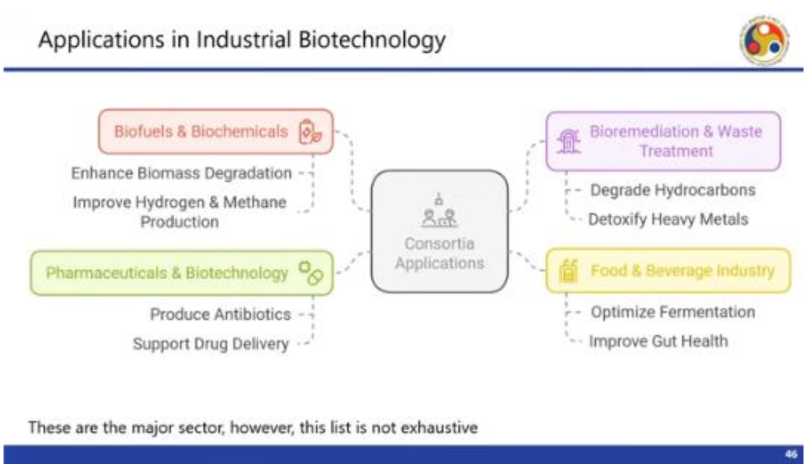


In dynamic flux balance analysis, while traditional FBA provides a static snapshot, dynamic flux balance analysis incorporates temporal changes, allowing for the simulation of microbial community dynamics over time. This approach is particularly useful for modeling environments where conditions change, such as in bioreactors or natural ecosystems. So, there are tools like the dynamic community FBA package that facilitate the implementation of dFBA in studying microbial consortia. Let us now move into the third section, where we discuss the applications of microbial consortia in industrial biotechnology. For example, in the food and pharmaceutical industries, in power generation with MFCs, and for bioremediation.



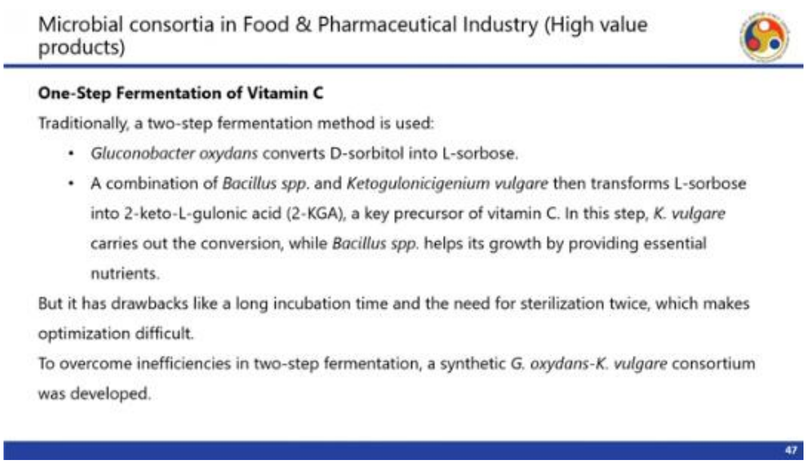
So, these microbial consortia have various applications. For example, in the production of biofuels and biochemicals, they enhance biomass degradation. They also improve hydrogen and methane production. In the pharmaceutical and biotechnology industry, they help produce antibiotics and support drug delivery. In bioremediation and waste treatment, they degrade hydrocarbons and detoxify heavy metals.

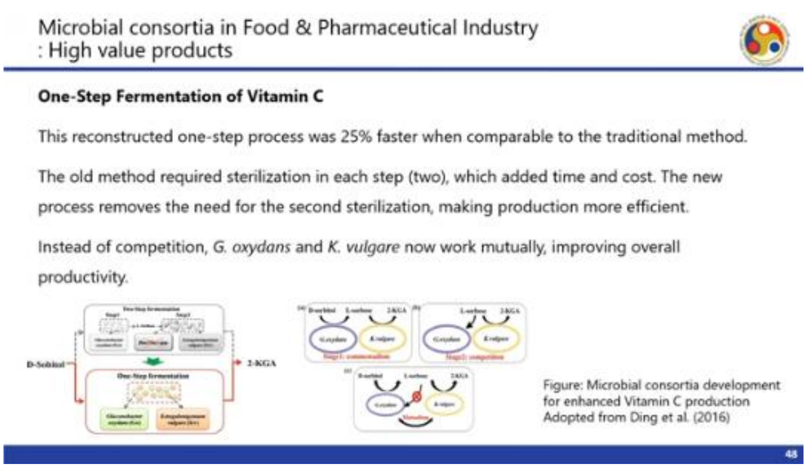
In the food and beverage industry, they optimize fermentation. They also improve gut health. These are some of the major sectors, but this is not an exhaustive list. There may be many other interesting applications of microbial consortia. Microbial consortia are used in the food and pharmaceutical industry, particularly for high-value products.



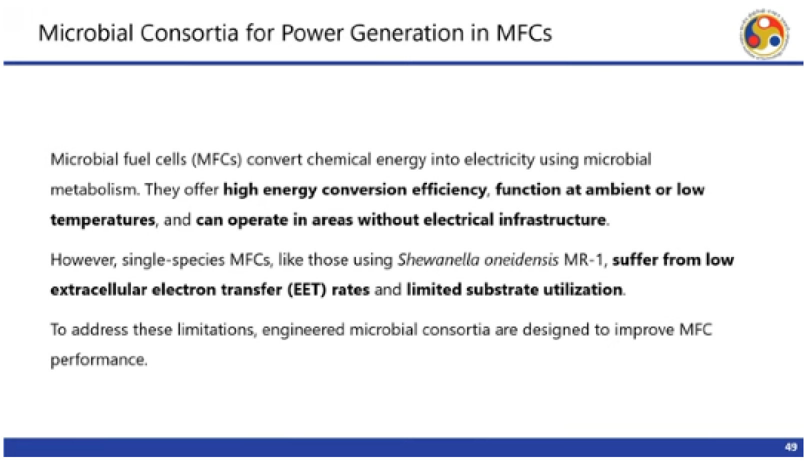
For example, we have the one-step fermentation of vitamin C. Traditionally, it's a two-step fermentation where Gluconobacter oxydans converts D-sorbitol into L-sorbose. A combination of Bacillus species and Ketogulonicigenium vulgare then transforms L-sorbose into 2-keto-L-gulonic acid, which is a key precursor for vitamin C. In this step, K. vulgare carries out the conversion while Bacillus species helps its growth by providing essential nutrients. But this two-step system has a drawback. For example, it requires a long incubation time and needs sterilization twice, which makes optimization difficult.

To overcome inefficiencies in the two-step fermentation process, a synthetic G. oxydans-K. vulgare consortium was developed. This reconstructed one-step process using these consortia was 25% faster than the two-step method used earlier, which is the traditional method. The old method required sterilization at each step, which took time and cost; the new process removes the need for the second sterilization, making production more efficient. Instead of competition, G. oxydans and K. vulgare work mutually, improving overall productivity. Then, the application of microbial consortia for power generation in microbial fuel cells converts chemical energy into electricity using microbial metabolism.



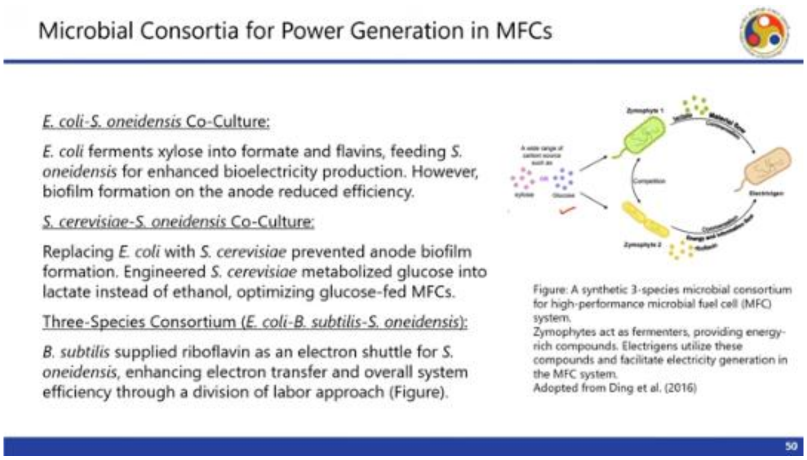


They offer high energy conversion efficiency, function at low ambient temperatures, and operate in areas without electrical infrastructure. However, single-species MFCs, like those using Shewanella oneidensis MR-1, suffer from low extracellular electron transfer rates and limited substrate utilization. To address these limitations, engineered microbial consortia are designed to improve microbial fuel cell performance. Here, in this figure, we see a synthetic three-species microbial consortium for a high-performance microbial fuel cell system. Zymophytes act as fermenters, providing energy-rich compounds.



Then, we have these electrogens, which utilize these compounds and facilitate electricity generation in the MFC system. Here, we have Zymophyte 1, Zymophyte 2, and electrogens. Together, they form the three species of this microbial consortium. Here, you have these E. coli and S. oneidensis co-culture, with E. coli fermenting xylose into formate and flavins, feeding S. oneidensis for enhanced bioelectricity production.

However, biofilm formation on the anode reduces efficiency. Then, we have the Saccharomyces cerevisiae and S. oneidensis co-culture replacing E. coli with Saccharomyces cerevisiae, which prevented anode biofilm formation. Engineered Saccharomyces cerevisiae metabolized glucose into lactate instead of ethanol, optimizing glucose-fed MFCs. Then comes the three-species consortium: E. coli, Bacillus subtilis, and S. oneidensis. Here, Bacillus subtilis supplied riboflavin as an electron shuttle for S. oneidensis, enhancing electron transfer and overall system efficiency through a division-of-labor approach, as already shown here in this figure.



Then, there are also other very important emerging applications of microbial consortia, which are trying to address current challenges, such as the degradation of plastic, which is now a huge environmental issue. Coa et al. reported a synthetic microbial consortium designed using a bottom-up approach, with each strain assigned a specialized metabolic function. In general, microbes like B. subtilis, Pseudomonas putida, and Rhodococcus enhance degradation efficiency by expressing plastic-degrading enzymes. This modular strategy minimizes metabolic burden on single strains, improving robustness and efficiency.

Converting plastic breakdown products into valuable bioproducts, such as biofuels or biopolymers, through engineered metabolic pathways is now the recent trend in research. So, you have these plastics, which may be PET, polyethylene, PVC, PU, or PP. Then you have this strain 1. So, this will attack the plastics and undergo biodeterioration. Then, we have these further released into much smaller fragments by strain 2.

So, this is an overall scheme of degradation of plastics by synthetic microbial consortia and then we may have other strains 3, 4 and 5, each of them doing different specific tasks and thereby degrading the polymers into different monomers and also in the process releasing water and carbon dioxide gas. So, let us now look into the recent advances on microbial consortia for bioremediation. So, let us look into the various substrates like plastic, petroleum, hydrocarbons and antibiotics and what are the achievements in the bioremediation front using microbial consortia. So, plastics like PET has been seen that there is a weight loss of polyethylene terephthalate, which reaches around 23.2% in seven days. And for these, co-culture of Rhodococcus pseudomonas putida and two metabolically engineered bacillus subtilis species has been achieved.

Then in the case of polyethylene, there was a demonstration of around 81% reduction for LDPE strips over 120 days. And enterobacter species, Bengaluru, and then enterobacter species, Bengaluru, btdsce01, 02, and of course, 03 and also pantoea species were used in this degradation. Then there is the polystyrene, which demonstrated 12.4% weight reduction and 23% weight loss of hips film in 30 days, utilizing bacillus species and pseudomonas species. Then in the case of polyurethane, around 50% of proprietary aromatic PE PU-A coat polymer was consumed in 25 days

when a co-culture of rhodobacteals, rhizobiales, burkholderiales, and then actinomycetales and sphingobacterials were deployed. Then in the case of petroleum hydrocarbons and N-alkane synergistic rate of diesel oil biodegradation was achieved 85.5% utilizing Pseudomonas stutzeri and Dietzia species. Then in the case of polyacrylic aromatic hydrocarbons, When sphingomonas, pseudomonas, sphingobium, dokdonella and luteinomonas were used, it was seen that there was a near complete degradation of fluorine and phenanthrine after 5 days.

Experiments on antibiotic degradation and bioremediation have also been conducted, where sulfonamide antibiotics were found to be degraded up to 78% after four weeks by Firmicutes and Bacteroides when co-cultured with these antibiotics. So with this, we come to the end of today's lecture. Thank you for your kind attention. Amen.

Recent advances on Microbial Consortia for Bioremediation

Substrate	Achievement	Co-culture strains
Plastic		
Polyethylene Terephthalate (PET)	The weight loss of PET film reached 23.2% in 7 days	Rhodococcus, Pseudomonas putida, and two metabolically engineered Bacillus subtilis species
Polyethylene (PE)	Demonstrated 81% ± 4% of weight reduction for LDPE strips over 120 days	Enterobacter sp. bengaluru-btdsce01, Enterobacter sp. bengaluru-btdsce02, and Pantoea sp. bengaluru-btdsce03
Polystyrene (PS)	Demonstrated 12.4% weight reduction of PS, and 23% weight loss of HIPS film in 30 days	Bacillus spp. and Pseudomonas spp.
Polyurethane (PU)	50.3% of proprietary aromatic PE-PU-A copolymer consumed in 25 days	Rhodobacterales, Rhizobiales, Burkholderiales, Actinomycetales, Sphingobacteriales
Petroleum hydrocarbons		
n-alkane	Synergistic rate of diesel oil biodegradation was 85.54% ± 6.42%	Pseudomonas stutzeri, Dietzia sp.
Polycyclic aromatic hydrocarbons	Nearly completely degraded fluorene and phenanthrene after 5 days	Sphingomonas, Pseudomonas, Sphingobium, Dokdonella, and Luteimonas
Antibiotic		
Sulfonamide antibiotics	78.3% degraded after 4 weeks	Firmicutes and Bacteroides, represented by Bacillus and Flavobacterium