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# VIDURAVA

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## Living Microbes: An Underutilized National Wealth

# VIDURAVA

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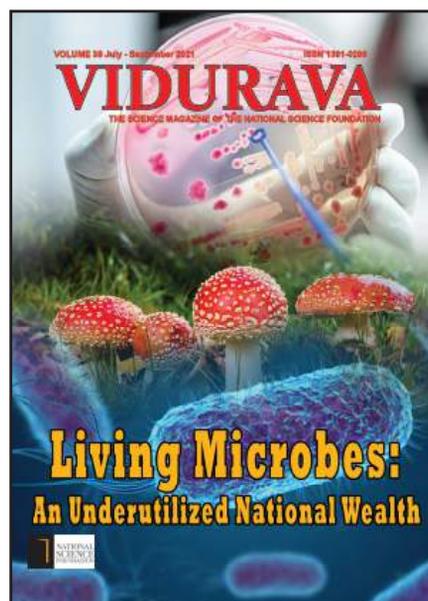
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# Editorial

## Microorganisms in the Service of Mankind

The current number of *Vidurva* presents a valuable and fascinating set of articles on a theme that had not been previously subject to discussion.

Microbes as one may note from these presentations, appear as a unique class of living organisms which have played a significant role in the life and times of *Homo sapiens*. In fact these organisms appear to be indispensable for the continuation of life forms on earth, which apparently they had themselves been responsible for its creation. Although some of the subject matter in these articles may be beyond the comprehension of the traditional reader of this magazine, what may seem to be insensible is why this precious gift of nature failed to reach the limelight it deserves. As of now most of the developments and knowledge on the vast capabilities of microbes appear to be in the hands of research scientists, who may themselves be examining the potentials and possible applications of their findings in economically feasible process technologies.

In this context it is interesting to note with expectations the physico-chemical possibilities, and the basis of transforming the virtually insoluble Eppawela Rock Phosphate deposit to a soluble and economically usable phosphate fertilizer with the active involvement of specific microbes.

Another significant observation is the use of microbes in the formulation of “Next Generation Biofertilizers”,. The Editor, as a former research scientist attached to the plantation sector, acknowledges with appreciation this fascinating research study on the potentials of transforming the traditional system of agriculture to new heights through the application of microbe – generated biofertilizers.

The team of scientists participating in this project have, in fact coined a new expression called “Omics” which seems to be a tool or mechanism that involves the simultaneous or integrated application of the processes referred to by these authors as metagenomics, metatranscriptomics, metaproteomics and metabolomics, all of which had not been in common use in the past. Although the contents of this impressive article may appear beyond the understanding of the lay reader of this magazine, it deserves the recognition and appreciation as a pioneering effort to explore a hitherto feebly understood field of science. It is likely that the final outcome of these investigations may even receive global recognition as a new pathway towards environment- friendly agriculture.

**M. Asoka T. De Silva**

## Living microbes: An invaluable global wealth

Vidya Nidhi S. A. Kulasooriya



Among all life forms on planet earth, microorganisms which are invisible to the naked eye, are the most abundant, most widely distributed and are indispensable for the continuation of life, which they themselves originated.

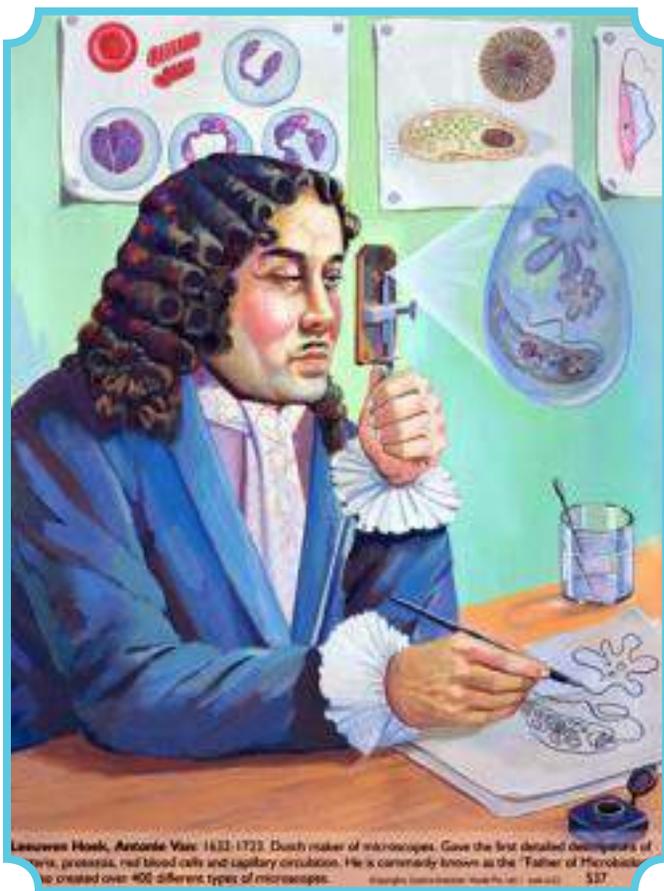
The discovery of microorganisms by Anton Van Leeuwenhoek (1632 – 1723) brought about a revolution in science. The immediate reaction to this discovery was confusion among taxonomists at that time, because all living things had been grouped either as plants or

animals. It was not possible to include microorganisms in either group because, they not only showed characters common to both but also possessed features unique to them and certain taxonomists called them “Chaos”. With the accumulation of knowledge on their morphology, physiology, biochemistry, genetics and molecular biology,

it became evident that microorganisms are an extremely diverse group with only one feature in common, that is, their minute size which make them invisible to the unaided eye. The discovery of these organisms led to a new discipline of science termed **Microbiology**: the study of microorganisms.

Pioneering studies in microbiology were done by well known European scientists mostly during the 19<sup>th</sup> century. Landmark studies in medical microbiology pioneered by Louis Pasteur, Ferdinand Cohen and Robert Koch (founders of bacteriology), the discovery of filterable viruses by Beijerinck, and the work of many plant and animal pathologists demonstrated that microorganisms are the causative organisms responsible for contagious diseases. It was also reported that most pathogens of plants and animals were also microorganisms. All this made microbes to be considered as dreaded germ's and several methods and materials were developed to destroy them.

These attitudes changed with the realization of the vital roles that



Leeuwenhoek, Antonie Van (1632-1723). Dutch maker of microscopes. Gave the first detailed descriptions of sperm, protozoa, red blood cells and capillary circulation. He is commonly known as the "Father of Microbiology". He created over 400 different types of microscopes. (Image courtesy: [www.16th-century.com](http://www.16th-century.com))

could be played by microorganisms in the continuation of life on Earth, and several other beneficial activities. The initial suggestion of Humphrey Davy in 1836, that plants may fix atmospheric nitrogen, was demonstrated by Boussinghalt (1838) and Atwater (1885) for symbiotic  $N_2$ -fixation in legumes, and Jodin (1862) for free living microorganisms. Beijerinck (1901) demonstrated aerobic nitrogen fixation by *Aerobacter chroococcum* and sulfate reduction, a form of anaerobic respiration, and Sergei N. Winogradsky (1856–1953), an Ukrainian-Russian microbiologist, soil scientist and ecologist demonstrated anaerobic  $N_2$ -fixation in *Clostridium pasteurianum*, and also proposed the concept of cycling of nutrients. He also reported that *Beggiatoa* oxidized hydrogen sulfide ( $H_2S$ ) as an energy source. His research on nitrifying bacteria reported the first known form of chemoautotrophy, and how a lithotroph fixed  $CO_2$  to make organic compounds using chemical energy. These discoveries had important implications on the understanding of biogeochemical cycles. Also the improvement and application of biological  $N_2$ -fixation in crop production gave a special impetus for microorganisms. After centuries of research studies it is now accepted that biological nitrogen fixation is confined to certain prokaryotic microorganisms and their symbiotic associations. Alexander Fleming (1881 – 1955) British (Scottish) physician, microbiologist and pharmacologist's work resulted in the discovery of lysozyme (Fleming 1922), and isolated, identified and reported the first antibiotic penicillin obtained from the fungus *Penicillium* (Fleming 1929). The subsequent



preparation of a myriad of antibiotics from microorganisms, are landmark findings that changed the negative attitudes of society towards microorganisms.

Modern society therefore looks at microorganisms in a more balanced manner with due recognition to the important roles that they play in accepting both positive and negative impacts that they have on our lives. According to current thinking, nothing more than 1% of all microorganisms are detrimental to human lives, where as a significant proportion are beneficial and the vast majority are neutral waiting to be explored and utilized.

### Origin and Evolution

The origin of life on earth is believed to have commenced some 3.8 billion years ago (bya). Scientific evidence supports that during abiotic evolution the entities that gave rise to non-nucleated, prokaryotic microorganisms possessed only Ribose Nucleic Acid (RNA) as their genetic material. The  $O_2$  evolving Protocyanobacteria appeared around 2.8 bya. During the Paleoproterozoic Era (2.5 to

1.6 bya), the earth began to cool down followed by continuous torrential rain. These led to the proliferation of cyanobacteria in the marine phytoplankton which fixed and stored part of the carbon and released oxygen ( $O_2$ ) that reduced atmospheric methane through oxidation. Meanwhile the chloroplasts that evolved through processes of endosymbioses between cyanobacteria and heterotrophic eukaryotic ancestors accelerated release of  $O_2$  to the atmosphere. Intense solar rays bombarding the earth converted some of the  $O_2$  to ozone ( $O_3$ ) which buildup a protective  $O_3$  layer that enabled the evolution and migration of life forms from the oceans to land. The oxygenation of the atmosphere also triggered off the development of the aerobic process of respiration. Respiration was far more efficient than the anaerobic processes of fermentation, and the aerobes proliferated at a dramatic pace driving the anaerobes to near extinction. Today aerobes are the dominant living forms among both flora and fauna thriving under an atmosphere containing 21% of oxygen. These evidences clearly show the crucial roles played by

ancestral microorganisms, and if not for them all other life forms that we see today may not be there. Microorganisms are ubiquitous in their global distribution occupying all the nooks and corners of the planet earth including extreme habitats such as hot springs, hypersaline localities, freezing environments and arid deserts. These versatile abilities and the intimate relationships including endosymbiosis they exhibit with all other forms of life are reflections of their pioneering ancestry. For 2.2 billion years they had been the only occupants of the primitive Earth. During this very long period microorganisms would

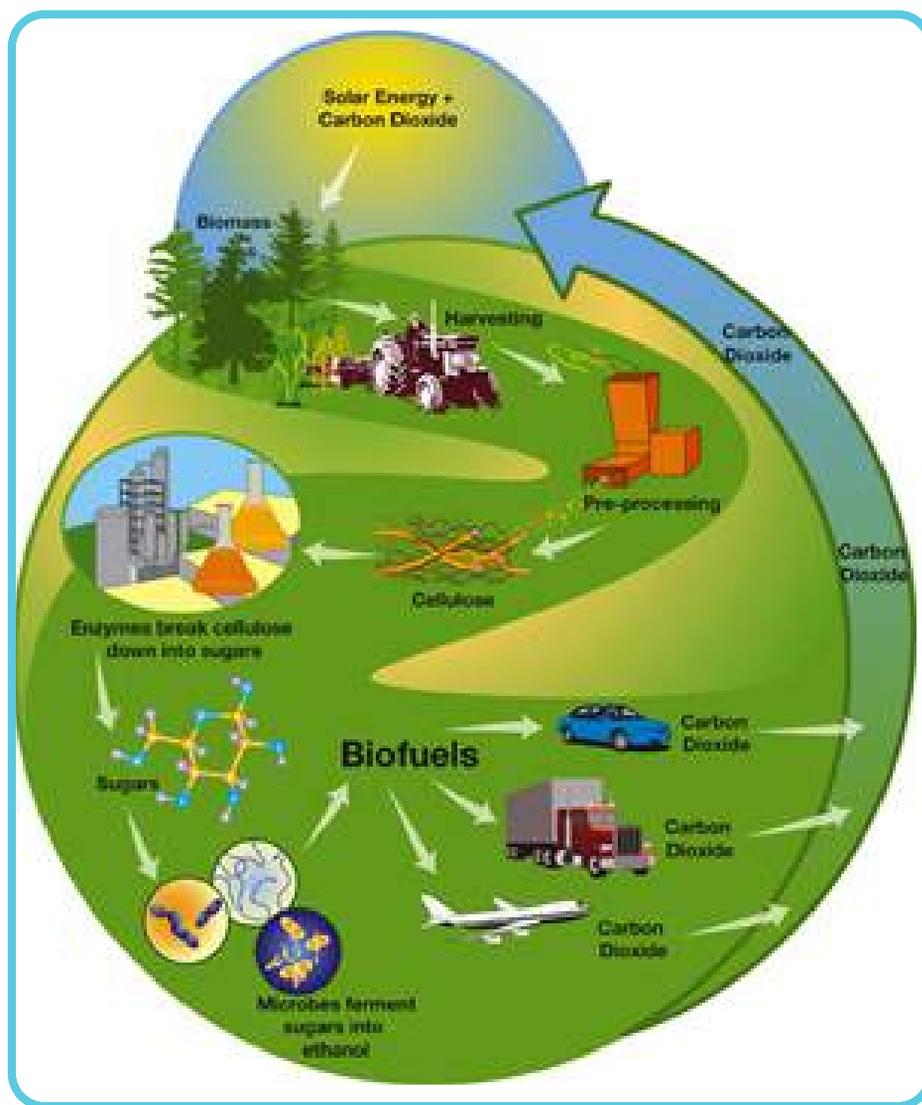
have developed mechanisms to utilize all the abiotic resources available on earth. This vast microbial biodiversity possessing an invaluable germplasm is a natural **biowealth** available for man to study, discover and utilize for all his needs and requirements. Therefore it is also not surprising to find intimate associations of microorganisms including endosymbiosis with all other living beings (plants, animals and man) because all of them have evolved in habitats teeming with microbes. It is alarmed to learn that the microbial cell population within us is more numerous than all the cells in our body tissues.

The widespread symbiotic relationships between microorganisms and all other living organisms on earth had given rise to a novel theory of ‘Symbiogenic Evolution’. This concept suggests that besides mutation, genetic recombination and natural selection, evolution would have involved symbiotic associations to form consortia of new structural dimensions. According to this theory most eukaryotic organisms are really consortia of organisms functioning in harmony towards common goals of development and adaptation. It has been reported that soil microorganisms seldom associate as individuals

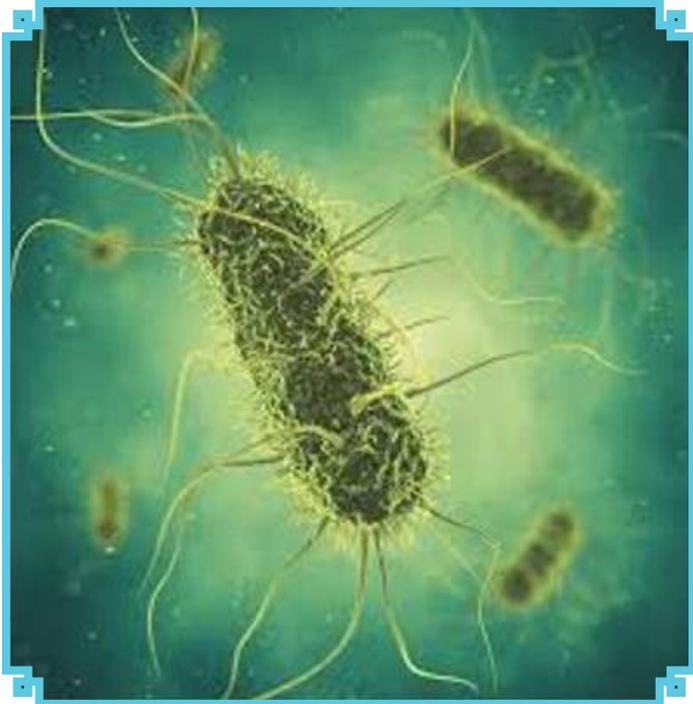
with roots of higher plants, but more so as consortia of multi functional microorganisms. Such root associated microbial communities have been termed **rhizosphere microbiomes**. Recent research studies on the preparation of novel biofertilizers, are focussed more on the development of multi-microbial inoculants to establish more efficient rhizosphere microbiomes.

### Uses of Microorganisms

Microorganisms are the primary agents of decomposition of organic matter, an exclusive process that enables the continuation of life on earth. They may have been used for thousands of years (even prior to their discovery) in the traditional practices of brewing alcoholic drinks and making bakery products. Subsequent to their discovery, microorganisms were used to elucidate basic processes of life such as photosynthesis, respiration,



metabolism, reproduction and genetics, because they can be cultured under controlled conditions to obtain genetically and metabolically uniform populations. In biotechnology and



molecular biology including genetic engineering, microorganisms are used as tools of genetic exchange. Today some of these organisms are selected, sometimes genetically modified and utilized extensively in the pharmaceutical and nutraceutical industries, as well as in the manufacture of dairy products, preparation of bio-fertilizers and bio-pesticides, production of biofuels, eco-friendly agents of sewage treatment, cleansing of oil spills, extraction of valuable metals, and in the development of microbial-fuel cells. Most of these uses can be exploited significantly in developing countries like Sri Lanka particularly for eco-friendly, sustainable activities.

On the negative side there are possibilities to develop genetically

modified, more virulent plant and animal pathogens including those against human being. The dreadful Corona viruses that we are experiencing today is a classic example, but so far there is no

unequivocal evidence to show that it had been produced intentionally. It is absolutely necessary that we become aware of such potential dangers of bio-terrorism and biological warfare. There are however international agreements, conventions and agencies to monitor, detect and

control such nefarious activities.

From the foregoing it is evident that the 'Microbial World' presents an array of organisms with a very wide range of diversity, and presents an invaluable gene pool which originated around 3.8 billion years ago. This germplasm had undergone evolution, natural selection and adaptation in harmony with all the changes undergone by the earth. These evolutionary processes are continuing incessantly, and it is most likely that the best adapted organisms among all living beings against the impending climate changes would be microbes. There is much more to be discovered among this fascinating biowealth, and it is our foremost

responsibility to protect this storehouse of invaluable genes for future utilization. It is also vital that we protect our endemic microflora because there are millions of organisms waiting to be explored and utilized. Recently two pharmaceutical products have been patented overseas and released under the names 'Lankamycin and Lankacidin' from bacteria isolated from Sri Lankan soils. We should all be alert against such exploitation of our endemic microorganisms, and laws have to be strengthened and implemented with vigour to prevent this type of bio-piracy. The most practical way to protect microbial biodiversity is the conservation of their natural habitats and ecosystems.



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# Use of microbes in next generation biofertilizers

Dr C. Bamunuarachchige, Mr H.K.S. De Zoysa and Dr P.N. Yapa



## Introduction

Biofertilizers are essentially microbes, composed of bacteria, fungi and blue-green algae that facilitate availability of nutrients for plant growth (Figure 01). Although biofertilizers have been used for a very long time, they have had very little success in replacing chemical fertilizers due to many challenges. However, with the ever-growing concerns of environmental pollution, negative effect on beneficial organisms and their involvement in human and animal

health issues, has prompted to look for more improved and efficient biofertilizers that can reduce the usage of chemical fertilizers. Hence, the challenges facing the next generation biofertilizers could be listed as follows:-

- 1.Improving the efficiency of microbial inoculants such as plant growth promoting rhizobacteria (PGPR) , and plant growth promoting fungi (PGPF).
- 2.Use of microbial consortia to provide multiple benefits.
- 3.Improving stability of inoculants

- during transport and storage.
- 4.Formulation for maximum survival.
- 5.Providing a stable environment for improved efficiency.
- 6.Use of uncommon inoculants such as extremophiles and micro algae, and the development of customized biofertilizers to suit the conditions of the fields and their geographical locations.
- 7.Identifying and popularizing other characters of biofertilizers in order to use them for bioremediation, improved plant physiology, and degradation of pesticides.

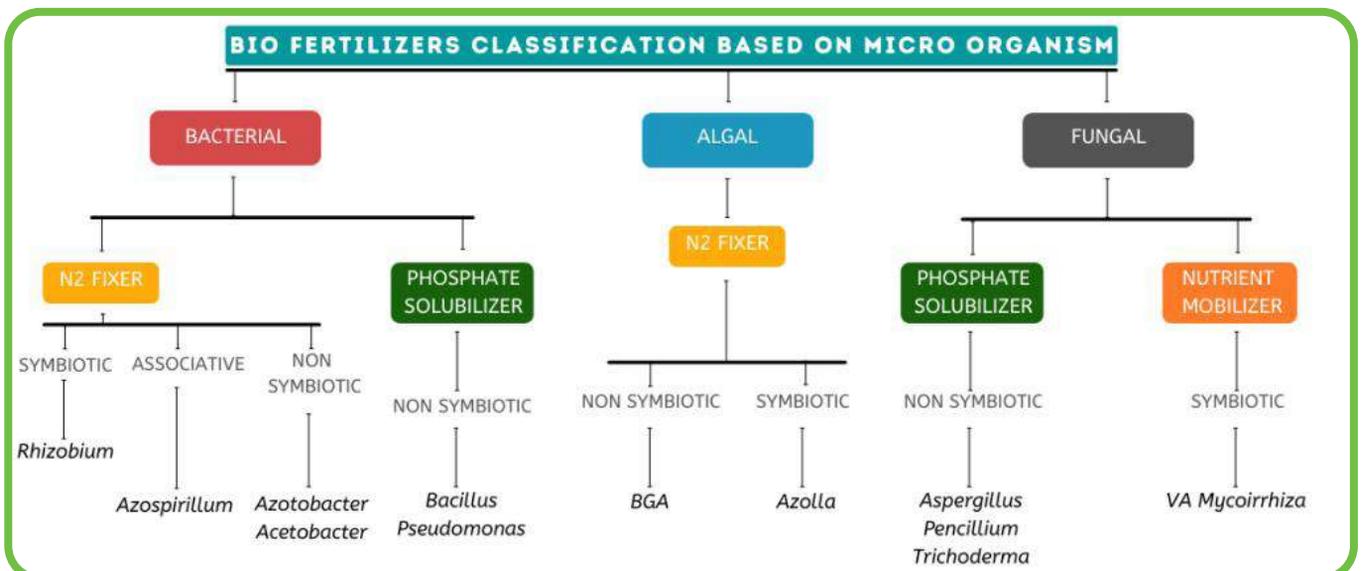


Figure 01: Biofertilizer classification based on microorganisms

With the rapid progress of technologies in molecular life sciences, these challenges can be supported by new integrated “omics” strategies, which include simultaneous use of metagenomics, metatranscriptomics, metaproteomics and metabolomics. Facilitated by ever increasing bioinformatic tools, the integrated omics has become the current tool in producing the next generation biofertilizers (Figure 02).

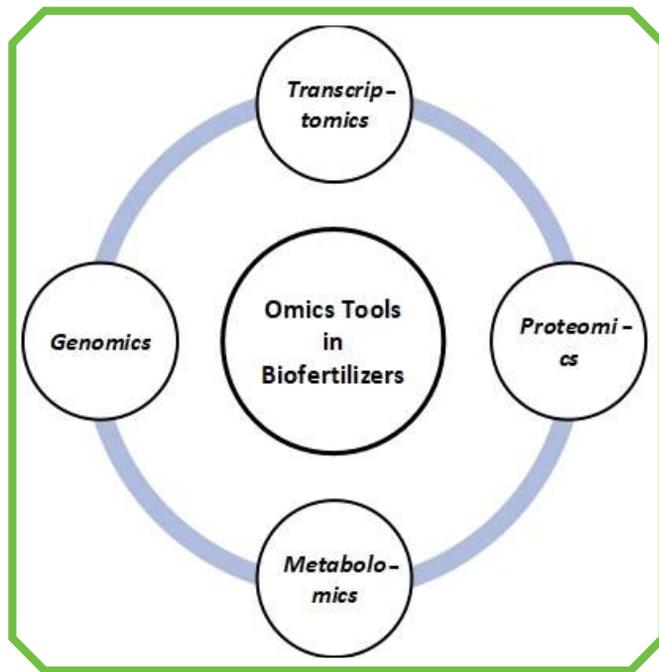
### Use of Omics Tools in the Development of Efficient Biofertilizers

The emerging omics technologies which include Next Generation Sequencing (NGS), microarrays and other chip-based approaches

fertilizers. These technologies have concurred some of the usual challenges such as identification of mixed and unculturable strains, genetic variants, understanding complex pathways, and the signalling processes in inter species interactions. Tools of omics can play a role at several stages in the production of biofertilizer and these include;

1. Identification and selection of strains of the plant microbiome
2. Investigating the respective biochemical pathways
3. Improving the strains for more efficient performance
4. Genetic engineering of plants for better response

### Identification and Strain Selection for Biofertilizers



**Figure 02: Different types of omics approaches for biofertilizers**

Whole Genome Sequencing (WGS) and RNA sequencing (RNA Seq), help to identify genes responsible and gene networks, including regulatory elements and metagenomics that facilitate massive amount of parallel sequencing. However, an in-depth understanding provided by specific genes, play

an important role in culture selection for efficient biofertilizers. Therefore, some genomic strategies allow identifying of all the responsible microbes and the

others select the best microbes for successful biofertilizers.

Metagenomics is the primary strategy for identifying all potential microbes in habitats that could expand the microbes in the biofertilizer industry. Inoculant strain development begins with the capture and identification of single strains or consortia (co-cultivated or co-inoculated microbial cultures). However, the success of these inoculants in commercially viable biofertilizers depends on their traits. These traits can be grouped into five levels;

1. Capture and refinement
2. Production
3. Establishment
4. Function,
5. Downstream impacts

Although the functional traits are important, establishment of microbes, which are more ecological, is equally important for efficient biofertilizer production. Henceforth, ideal microbial strains should possess both functional and ecological characters. The conventional culture-based capture methods may not pick up strains good at establishment and persistence. Moreover, many unculturable strains may possess ecological characters that make them superior in the environment as biofertilizers. Metagenomic tools of studying microbiomes include sequence-based metagenomics and functional metagenomics. The outputs of functional genomics contribute to strain selection and strain improvement, and improving plant response to microbes while the sequence-based metagenomics help to capture total strains in the rhizosphere. The combinations of these approaches can identify microbes for the next generation

have revolutionized the field of agriculture with the hope of converting conventional biofertilizers into precise, efficient, and reliable alternatives to chemical

biofertilizers, which are both functionally important and ecologically stable.

### **Sequence Based Metagenomics in the Capture of Potential Strains for Biofertilizers**

The basic product of the sequence-based metagenomics approach in next generation biofertilizers, is the identification of microbial taxa in the rhizosphere samples to the genus or species level. Many parallel techniques have been developed under sequence-based metagenomics, which could be categorized into either targeted gene sequencing, or WGS. The prominent target gene sequence based metagenomic tool for bacteria is the 16S rDNA, because of the conserved sequences. For fungi, it is the Internal Transcribed Spacer (ITS) sequences.

Assigning taxa after a metagenomic analysis is done using a bioinformatic software and reference data base. For example, a metagenomic study conducted on soil microbial population and enzyme activities using MiSeq platform, used MiSeq Reporter for preliminary data analysis with Qiime, for assigning taxa at the species level based on Greengene database for bacteria and UNITE a web based database and sequence management environment for the molecular identification of fungi .

### **Screening of Potential Strains from Other Habitats**

While it may be too early to think that cultures isolated from extreme niches could be developed into next generation biofertilizers, the fact that they may play a role in strain improvement either through genetic

engineering or gene editing, cannot be ignored. Moreover, customized biofertilizers for geographic areas and temperature regimes may be a reality soon, where soils without ideal conditions for cultivation may become the norm. Under these circumstances, soil reclamation may become the first step of microbial inoculant intervention. Such inoculants could also be used more as strains facilitating bioremediation rather than biofertilizers in contaminated soils. For instance, a rhizosphere metagenomic study conducted in oil-contaminated soil has revealed the significant presence of hydrocarbon degrading microbes.

### **Use of Extremophiles as a Prospective Source of Biofertilizer**

Hyperthermophilic ( extreme high temperature loving), thermophilic (high temperature loving), psychrophilic (cold loving), and halophilic (salinity loving) microbes could be considered as the extremophilic microbes. Conventionally, research on extremophiles have been dependent on culture-based techniques. However, with the introduction of metagenomics, this area of research has seen a massive expansion. A study on western deserts of Himalaya has identified many cold loving microbes with plant growth promoting (PGP) capabilities, under cold conditions. These capabilities include many properties ideal for next generation biofertilizers including; phosphate solubilization, aminocyclopropane -1- carboxy)ate (ACC) deaminase activity, production of molecules such as Indoleacetic acid (IAA), gibberellins, and production of siderophores. A metagenomic

research on sediments of hypersaline Siberian soda lakes, identified microbes belonging to 45 phyla including 5 new species belonging to Candidate Phyla Radiation (CPR) and novel dominant members in previously identified groups of C, N and S cycling bacteria. Acidophiles (acid loving) and acid tolerant microbes are another source that may become beneficial in next generation biofertilizers, especially when some cultivable lands, such as rice fields have already become acidic. Cultures isolated from peat swamp forests of Southern Plant Growth Promotion (PGP). Thailand have shown several PGP traits such production of IAA, ALA, siderophores, phosphate solubilization and N<sub>2</sub> fixation at below 5pH range indicating the importance of this group in next generation biofertilizers. Although targeted sequence metagenomics identify the potential microbes that could be used as inoculants for biofertilizers, it can only confirm the taxa involved. The true potential can only be investigated via the techniques of WGS, transcriptomics, proteomics, and metabolomics. While all these techniques could individually offer valuable information for selection of strains, it has become the new wave to use the integrated omics approach due to the ability of cross referencing between the technologies and the better prediction capabilities.

### **Use of Transcriptomics, Proteomics and Metabolomics in Microbial Strain Selection for Next Generation Biofertilizers**

Although WGS can provide valuable information about

the genome composition, gene clusters and the functions of genes involved, further research at transcriptomic (mRNA), proteomic (protein) and metabolomic (metabolite) levels is required not just to confirm the predictions, but also to identify the expression levels, regulatory networks, and the metabolic profiles. Moreover, even when the relevant gene clusters could be identified by WGS, some microbial strains have not shown the expected PGP phenotypes. For instance, a research study on two plant-associated *Rhodospseudomonas palustris* strains (PS 3, and YSC 3) have indicated that only one strain was capable of PGP, even with very similar PGP gene clusters identified in both genomes. This proves the presence of the genes alone does not guarantee the functional roles of the genes. Besides, the gene expression for PGP microbes depend so much on the interaction with the relevant plant, maintained through chemical exudates released to the rhizosphere.

Metatranscriptomic approaches have become important to expand the basic information provided by metagenomics. Metatranscriptomics study the functional ecology rather than the annotated (predicted) ecology of the genomics. For instance, a study focusing on metagenomic and metatranscriptomic analysis of soil metagenomes clearly identified that although some strains are dominant in metagenomic analysis, functionally they may not be active.

Thus, metatranscriptomics provide information on how the expressions change temporally against changing environmental factors and interactions with other

organisms. Hence, together with metaproteomic and metabolic profiling, metatranscriptomic studies could help establishment and persistence of microbial strains and consortia used in next generation biofertilizers.

Metaproteomics, which is the investigation of microbial proteins by mass spectrometry (MS) could be of two basic types, the intact protein MS/MS or “top down” and shotgun or “bottoms up” tandem MS/MS working with peptides. Developments in metaproteomics have identified metabolic pathways of microbes which can be used effectively to select efficient cultures for development of inoculants for biofertilizers. The workflow used in metaproteomic studies can have a direct impact on the outcomes. A seawater metaproteomic study that compared gel-based and gel free protein fractionation methods with four different protein databases, clearly showed that the number of proteins, taxonomic structures and functions of the proteins varied with the type of workflow used. Based on this evidence, the experimental workflows need to be diverse for better metaproteomic analysis.

Metabolomics involve metabolic profiling or metabolic fingerprinting. Many techniques can be used in metabolomics, including nuclear magnetic resonance (NMR), time of flight mass spectrometry (ToF-MS), Fourier-transform infrared spectrometry (FT-IR), GC MS, HPLC, and ultra-high-performance liquid chromatography (UPLC). While identifying the metabolite profile allows to recognize various

PGP functional molecules such as growth factors and hormones, antibiotics, siderophores and many more, identifying these metabolites has also helped start a new trend in using metabolites in formulation with bioinoculants giving rise to more efficient next generation biofertilizers capable of surviving during transportation and storage.



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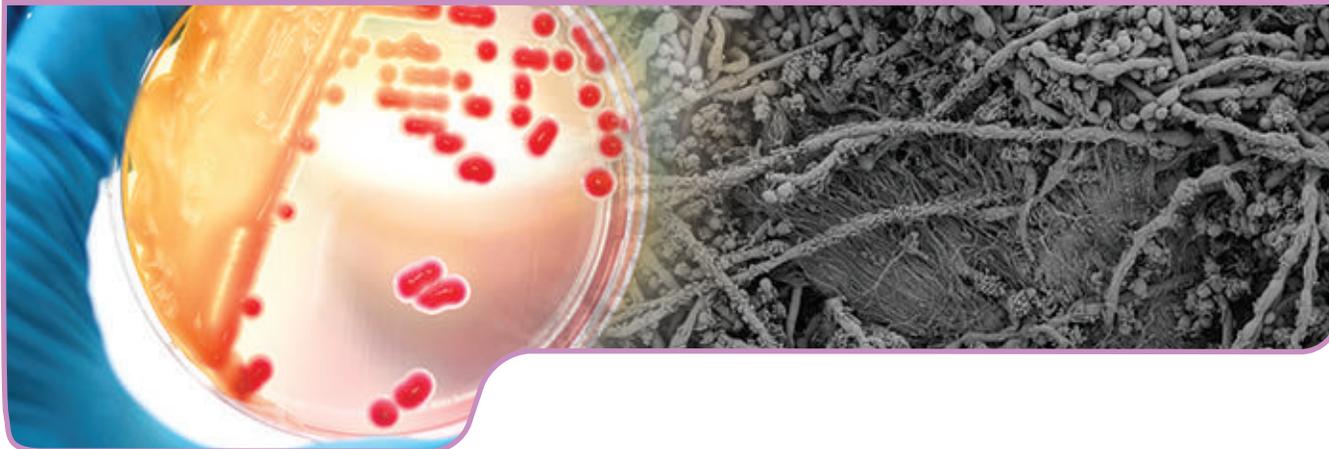


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## Phosphorous solubilizing microbes: A promising approach to minimise triple super phosphate usage in rice cultivation

Dr D.M.S.H. Dissanayaka and Mrs J.P.H.U. Jayanethi



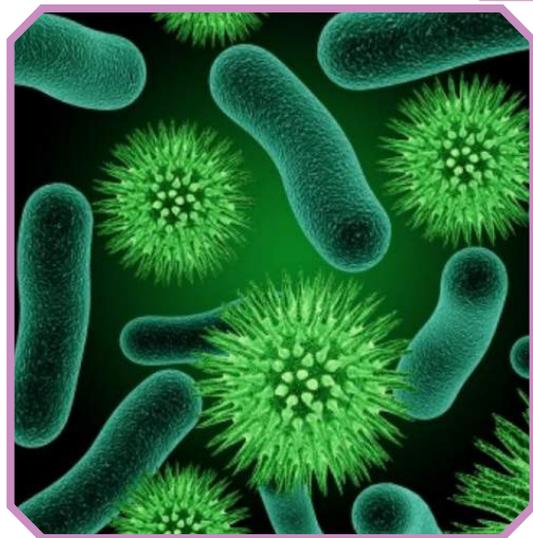
Phosphorus (P) is one of the major plant nutrients that lead to optimum crop growth and production. Even though most of the soils contain substantial P reserves, in most soils P remains insoluble, out of which only less than 10% is available for plants. Generally, available P concentrations are low in reddish-brown earth (RBE), which is the major soil group in the dry zone of Sri Lanka, where main agricultural production takes place. Previous studies revealed that the available P status of RBE varies from 1 to 87 mg/kg<sup>-1</sup>. The high P fixation in

soils leads to P deficiency in most Sri Lankan soils. Thus, the farmers use large quantities of P fertilisers to achieve the desired plant productivity.



more than 90 % of the P is water-soluble. Hence TSP could be rapidly absorbed by plants. Due to high water solubility, TSP is used as the P fertiliser for many crops, including annuals and perennials. Phosphate rock undergoes several acidulation processes in the TSP manufacturing process, leading to the addition of some trace elements into TSP. The trace element content of the TSP varies widely depending on the source of TSP.

It has been revealed that TSP applied rice fields in Sri Lanka have notable trace elements such as aluminium, chromium, nickel, cadmium, lead and uranium. According to literature, the Cd levels detected in TSP used in rice fields ranged from 23.50 to 71.74 mg/kg, which is higher than the maximum permissible levels of 10 µg g<sup>-1</sup> of the Sri Lanka Standards Institution (SLSI). Further, TSP contains a higher concentration of As (about 30 mg/ kg<sup>-1</sup>) than all the other P fertilisers used in Sri Lanka. The application of TSP increases the movement of Cd through food chains and can affect human health. The adverse effects of Cd on human health were first observed



Triple Super Phosphate (TSP) is widely used as a synthetic phosphate fertiliser in Sri Lanka (Figure 01). It is produced by the reaction of concentrated phosphoric acid with finely ground phosphate rock. Monocalcium phosphate is the active ingredient of TSP, and it is identical to superphosphate. It has a high P content, and

in subsistence rice farmers in Japan in the mid-1950s. They contracted a detrimental Cd poisoning (Itai-Itai disease) after consuming rice

and Fe and Al as impurities. The percentage of  $P_2O_5$  in ERP depends on the nature of the apatite crystals in the matrix, and the mining technique. The solubility of ERP is very low compared with other phosphorous fertilisers. Usually, citric acid solubility of ERP varies from 4 % to 6 % . Therefore, it is not recommended for short term crops or annual crops like rice and vegetables. ERP is generally used as a direct application for some perennial

since it causes only a meagre environmental impact on trace element accumulation. However, the application is limited due to its' low solubility. ERP is a good alternative for TSP if the solubility could be enhanced. In this regard, Phosphorus Solubilizing Microorganisms (PSMs) (Figure 03) play a vital role in solubilising phosphates in ERP.

The Phosphorous Solubilizing Microbes (PSMs) are a group of heterotrophic microorganisms capable of solubilising the inorganic forms of P by excreting organic acids. Naturally occurring PSMs were first discovered in 1903. They are highly concentrated in the rhizosphere, and they have higher metabolic activity than other microorganisms. Bacteria have a



**Figure 01: Triple Super Phosphate Fertilizer**

grown in soils that have been heavily polluted with Cd and other trace elements due to rapid industrialisation during the 1960s. The disease caused a softening of the bones and kidney failure. It is hypothesised that Cd accumulation in the human body causes Chronic Kidney Disease of Unknown etiology (CKDu), prevailing mainly in the dry and intermediate zones in Sri Lanka.

Eppawala Rock Phosphate (ERP) is a cheap, phosphate-rich source used as a P fertiliser. The ERP deposit was discovered in 1971 by the Geological Survey Department of Sri Lanka (Figure 02). It is the largest rock phosphate deposit (approximately 40 million MT) in Sri Lanka, situated in Eppawala, in the North Central Province. It contains about 27 - 40 %  $P_2O_5$ ,

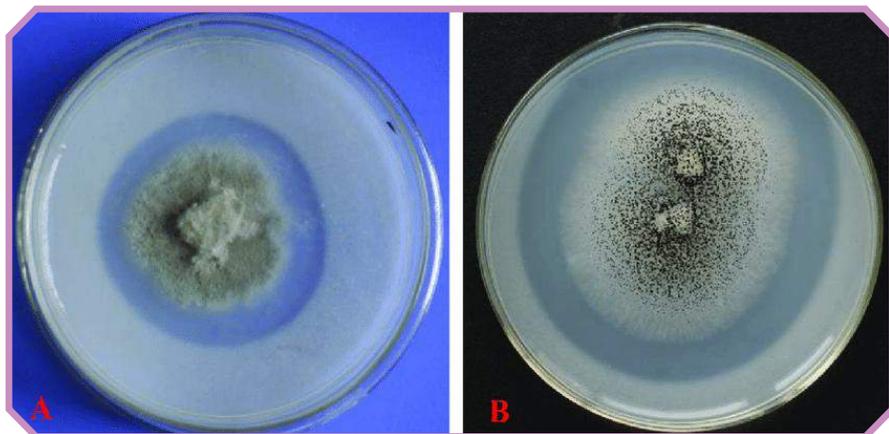


**Figure 02: Eppawala Rock Phosphate (ERP) Deposit**

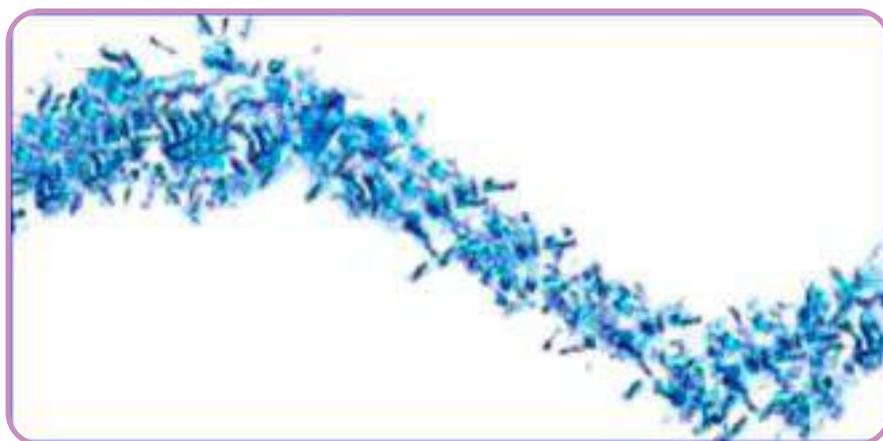
crops like tea, rubber and coconut, and some export agricultural crops grown in acidic soils. Further, ERP contains certain trace elements such as Cd ( $1.92 \mu\text{g g}^{-1}$ ) and As ( $8.56 \text{ mg/kg}^{-1}$ ) in minute amounts.

Eppawala Rock Phosphate shows an excellent potential to be used as a P fertiliser for annual crops

higher potential than fungi to P solubilisation. Ectorrhizospheric strains such as *Pseudomonas* and *Bacilli*, and endosymbiotic rhizobia in soil bacterial communities have been identified as effective phosphate solubilisers. Among fungi, *Achrothcium*, *Alternaria*, *Arthrobotrys*, *Aspergillus*, *Fusarium*, *Glomus*, *Micromonospora*, *Penicillium*,



**Figure 03: Phosphorous Solubilizing Microorganisms (PSMs)**



**Figure 04: Fungal Bacterial Biofilm**

*Saccharomyces* have been identified as effective P solubilisers. Moreover, approximately 20% of actinomycetes can solubilise P, including those in the genera *Actinomyces*, *Micromonospora*, and *Streptomyces*. Cyanobacteria have also been reported as P solubilisers. Solubilisation of P by PSMs is carried out through various mechanisms and microbial processes such as organic acid production and enzyme secretions.

Most of the PSM communities exist in the soil as biofilms. A biofilm consists of microbial cells (algal, fungal, bacterial and/or other microbial) and an extracellular biopolymer known as an extracellular polymeric substance (EPS) produced by microbes providing the structure

and protection to the community (Figure 04). These communities can be found in different environments. Biofilms can be developed *in vitro* as bio-fertilisers, known as Biofilmed Biofertilizers (BFBFs). Applying BFBFs in rice cultivation has significantly reduced chemical fertiliser usage by about 50 %. Also, the use of BFBFs has no known adverse environmental impacts, eventually leading to sustainable agricultural systems. National Institute of Fundamental Studies (NIFS) has developed four biofilm formulations with the potential to solubilise ERP, and preliminary studies have shown promising results.

The environmental impacts and associated health hazards due to

the heavy usage of TSP can be effectively minimized by developing approaches to replace TSP with ERP, which contains minute amounts of trace elements. For example, if ERP can be applied with a suitable microbial biofilm containing PSMs, it would provide an excellent alternative to minimise the TSP usage in rice cultivation.



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## Cellulolytic microbes: Potential living resources for industrial applications in Sri Lanka

Ms S.K. Jayasekara



### Discovery of microbes: the emergence of an invisible world

The discovery of microbes dates back to 17<sup>th</sup> century. Simple microscopic observations, descriptions and depictions of protozoa and bacteria by Antoni van Leeuwenhoek; “the father of Microbiology”, and the initial illustrations of *Mucor*, a microfungus by Robert Hooke provided the most important preliminary insights on the existence of microbes. Since the discovery of this new world of microscopic inhabitants, scientists became more curious to learn further about these amazing living entities.

Initially, microbes were mostly known as accelerators of domestic food processing practices such as making bread, preparing wine, vinegar, toddy, pickles etc. and as causative agents of infectious diseases. Therefore, human–microbe interrelationship has a long history. Consequently, microbes were recognized as indispensable biological resources in more advanced

biotechnological applications. With the developments in science and technology, people were keen on finding out the underlying biochemical basis of these microbial processes. Accordingly, the role played by microbial enzymes was revealed. Currently, the worldwide recognition earned by microbial enzymes is enormous due to their widespread applications in various industries including agriculture, medicine, environmental conservation, bioenergy production, technical use, food manufacturing, animal nutrition, cosmetics, and as tools for research and development.

### Cellulolytic microbes: the decomposers of cellulose

Imagine, what would happen in a world without cellulolytic microbes? There may be plant debris, fallen leaves, branches or twigs piled up everywhere on the earth’s surface without decaying. so, how do these cellulolytic microbes digest and remove plant waste materials? To answer this question, it is necessary to understand the general structural makeup of plants. The major structural component

of plant debris is cellulose, because it is the substance that forms most of a plant’s cell wall. Since it is present in all the plants, cellulose is probably the most abundant complex polysaccharide on earth.

Cellulolytic microbes produce “cellulase” which is the enzyme that catalyzes the decomposition of cellulose; that to, their carbon and energy source. Being a multiple enzyme complex, cellulase sequentially acts on the cellulose polymer, and breaks down its  $\beta$ -1, 4-linkages. This cellulose digestion process produces easily fermentable sugar forms like glucose, cellobiose and simple oligosaccharides. However, the complete enzymatic hydrolysis and hydrolytic productivity of cellulose require the synergistic action of three major enzymes: endoglucanase, exoglucanase and beta-glycosidase. Different microorganisms produce different quantities of these enzymes in a complex which directly affect the cellulose digestion efficacy of individual microbes. In nature, microorganisms do not act individually on cellulose; rather they carry out these reactions

synergistically in order to attain their daily energy requirements in a sufficient way. This synergism makes cellulose hydrolysis a very efficient process in the natural environment. Unsurprisingly, making these cellulolytic microbes to perform the same natural cellulose hydrolysis process in an industrial setting would demand both theoretical and practical interventions of science.

### Microbial cellulases: current global trends

Cellulose could be utilized as a renewable and a cost-effective raw material in many industries through microbial cellulase. This is particularly the era during which the world makes an effort to implement sustainable development concepts. Hence, there is an enormous recognition

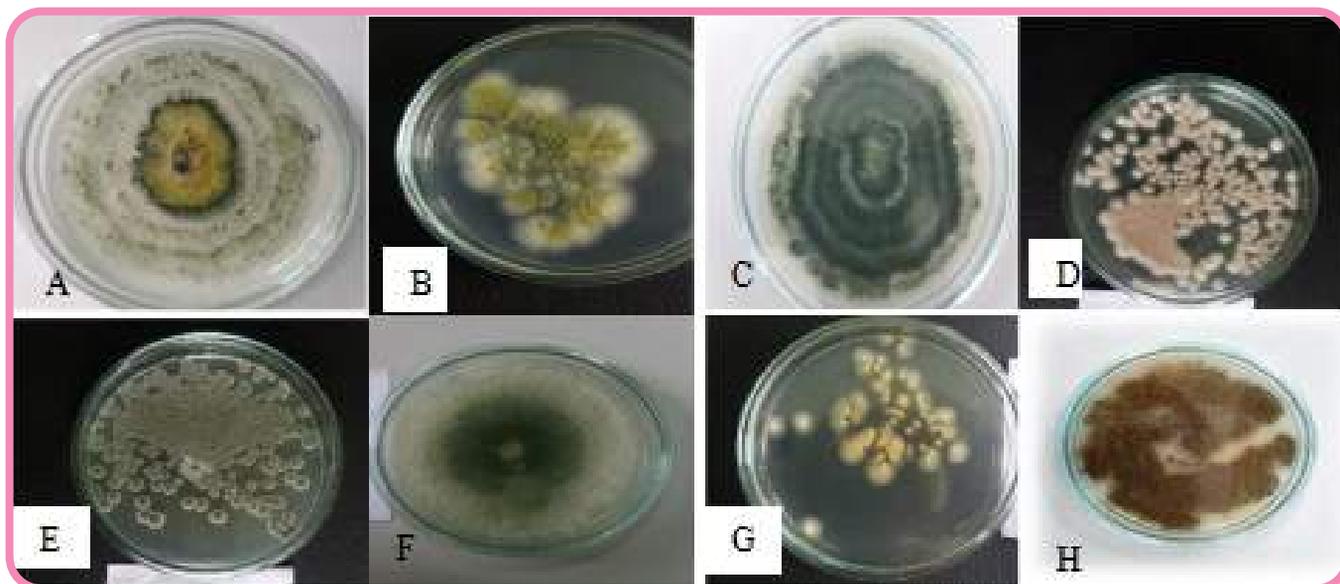
transformation of cellulosic biomass into sustainable biofuels is one of the emerging trends in the world. It is a promising alternative for fossil fuels as it would provide solutions to global increase in energy demand and depletion of fossil fuel stocks.

Using cellulases in the industrial sector however, is not a new concept. Cellulase has been commercially utilized for more than 30 years. In recent times the worldwide recognition earned by microbial cellulases is extensive due to their widespread applications in the textile industry for textile wet processing, bio stoning of denim fabric, biopolishing of textile fibers, softening of garments, removal of excess dye from fabrics, and production of laundry detergents. Additionally, cellulases are applied in pulp and paper industry, food

applications, protoplast production, genetic engineering and pollution treatment.

As cellulase is of microbial origin, even today, scientists all over the world are exploring the nature for more efficient cellulase producing microorganisms. There is a huge potential to extract the enzyme from these microbes, which could be applied in industries. A broad range of cellulases producing microorganisms including bacteria and fungi have been identified so far. They may be either aerobic or anaerobic microorganisms. However, aerobic microorganisms such as filamentous fungi are found to be more efficient in cellulase production.

Although an impressive collection of fungi such as *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp.,



**Figure 01: Locally isolated cellulolytic microorganisms.** (A). *Trichoderma* sp. (B) *Aspergillus* sp. (C). *Trichoderma* sp. (D). *Fusarium* sp. (E). *Penicillium* sp. (F). *Trichoderma* sp. (G). *Aspergillus* sp. (H). *Aspergillus* sp.

for ecofriendly, microbial processes of converting cellulosic biomass into energy, It also has contributed to the expansion of global cellulase enzyme market. Especially,

processing, brewing, plant fiber extraction, animal feed, agriculture, olive oil extraction, carotenoid extraction, pharmaceutical and medical sciences, analytical

etc., have been studied for being cellulolytic, *Trichoderma reesei* is one of the main fungal species which is broadly utilized in industrial scale cellulase production. They

produce an extracellular cellulase enzyme complex which makes it easier harvesting the enzyme in the downstream process. Anaerobic microorganisms, for example, bacteria like *Clostridium thermocellum* produces a cell-attached cellulosome which has the ability to directly attack crystalline as well as amorphous regions of cellulose. In the above context, discovering more efficient cellulolytic microbes could be a better approach of meeting the huge demand for the enzyme in the global market.

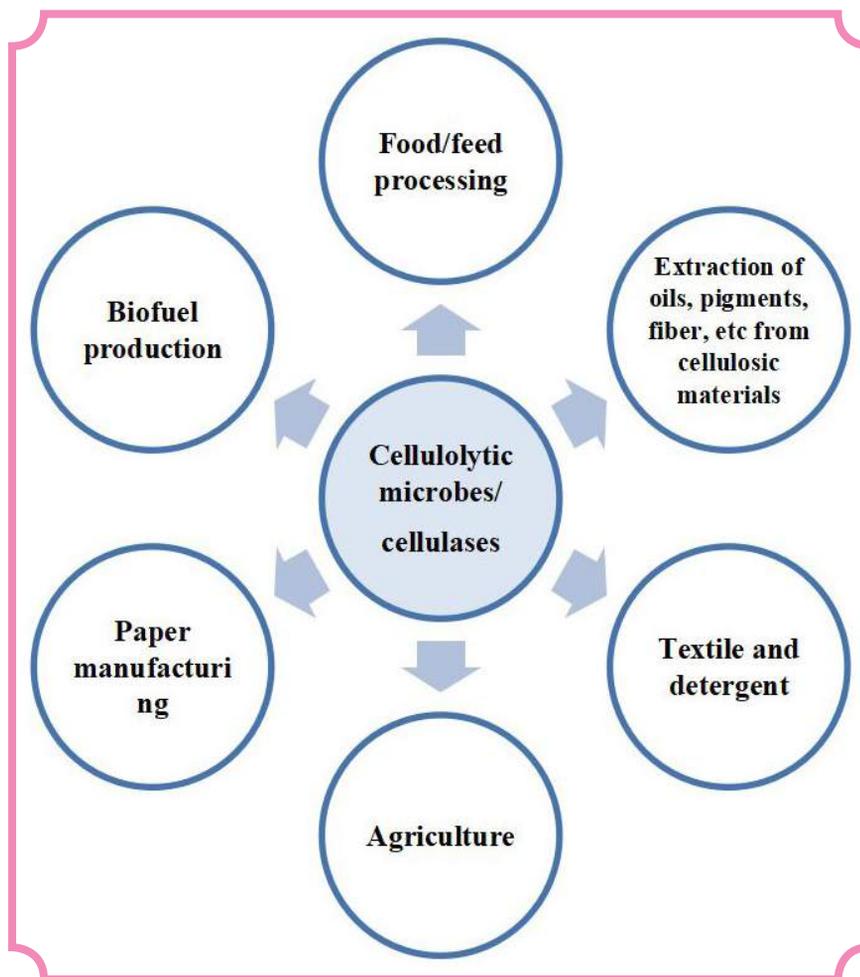
### Cellulytic microbes: The Sri Lankan context

Sri Lanka imports cellulase as it is an indispensable ingredient in the denim stone-wash or enzyme washing plants in the apparel industry. As a consequence, the industrial expenditure on cellulase imports is significantly higher. Hence the production of cellulases from indigenous microbes would be a remedial action to decrease this huge outflow of funds from the country on cellulase imports. Moreover, establishment of biotechnological strategies to produce cellulase locally is a possibility because of Sri Lanka's rich biodiversity. Our natural habitats, including tropical rain forests are teeming with cellulase secreting microbes. Unfortunately, they are an underutilized natural wealth.

Several Sri Lankan scientists have already initiated steps to explore the natural environment for cellulolytic microbes, and are evaluating their potential applications. Many scientific investigations have been conducted and are continuing on isolation, identification and

studying the possible applications of microbial cellulases. In the context of cellulolytic microbes and their enzyme systems, Sri Lankan scientists are expected to contribute greatly to further discoveries. It is essential to study

essential to enhance microbial cellulase production efficiency. Currently, there are promising molecular biotechnological approaches to enhance cellulase production by microorganisms. If developed to large scale

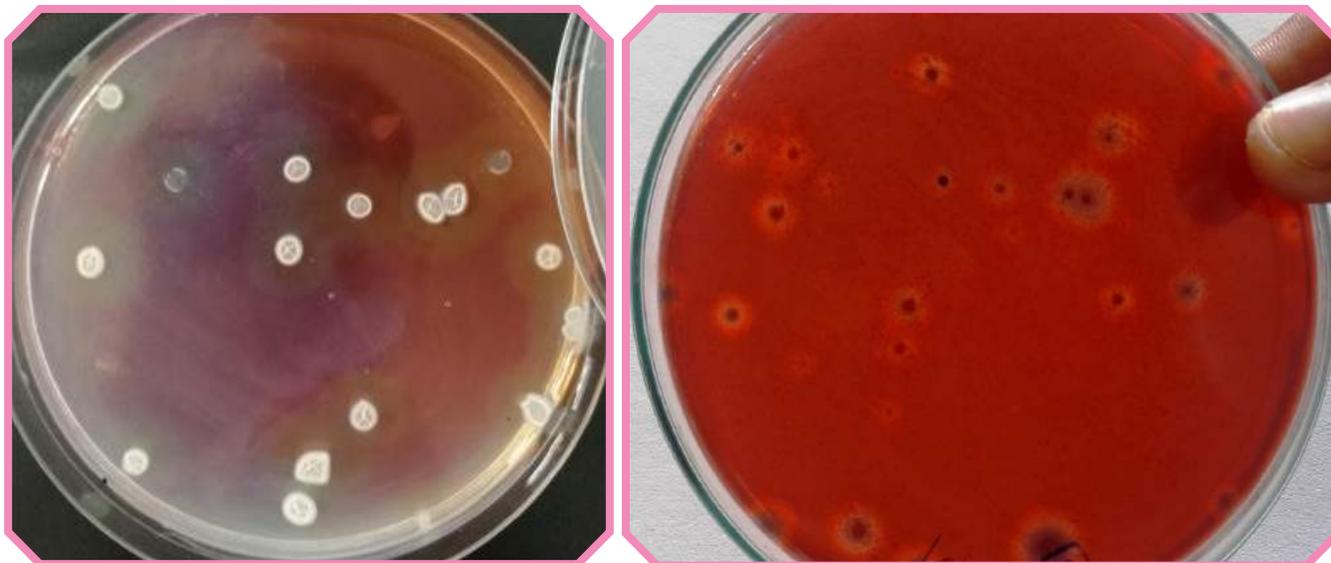


**Figure 02: Potential industrial uses of cellulolytic microbes**

diverse cellulase enzyme systems veiled by these microorganisms. In addition, well-organized application strategies (i.e. which include optimum reaction conditions, rate limiting factors, composition of cellulose, composition of cellulase, etc.) are required to utilize cellulase obtained from locally isolated microorganisms in industrially significant process. For this purpose, it is also

production it would open up new investment opportunities in the country beginning from cellulase production to a broad range of other industrial applications. They must also take initiatives to share the discovered knowledge with potential entrepreneurs, investors or industries that are capable of practically applying this knowledge.

Especially, as a solution for rising fuel demands in Sri Lanka,



the production and use of cellulosic biofuels could be popularized. Many countries in the world such as Brazil, USA, and Europe commercially use biofuels to fulfill their energy needs. Therefore, incorporation of these underutilized microbial resources into biofuel production is possible and it would be a great step forward in the advancement of Sri Lankan economy. However, it is impossible to ignore the fact that it is a time consuming process to establish a long lasting, well-functioning production process. It demands continuous research and development activities to maintain a productive industrial process.

Moreover, Sri Lanka has a rich history of cultivating their crops using organic fertilizer. Even without knowing the scientific mechanisms operating in the process, they traditionally used the natural microbial processes to add nutrients to their fields by incorporating cattle dung, paddy straw, rice husks, coir dust and leaves from different nitrogen rich plants like *Gliricidia* spp. Our ancestors increased

their harvests by applying these traditional agricultural techniques. Therefore, it is possible to use cellulolytic microorganisms in combination with other microbes such as nitrogen-fixing bacteria, and phosphate solubilizing bacteria to derive manure from cellulosic waste. However, returning to these eco-friendly microbial based agricultural systems cannot be achieved within a short period of time. At the same time, it is crucial to establish the most suitable, ecofriendly method that fits the modern-day agriculture. Moreover, the national paper manufacturing industry in Sri Lanka could be uplifted by introducing strategies to convert various cellulosic biomass into paper using locally isolated cellulolytic microorganisms. Additionally, locally produced cellulase could be used in local denim washing plants. Afore-mentioned are only a few examples for potential use of cellulolytic microbes in Sri Lankan industries. Apparently, there is a huge potential of utilizing locally isolated cellulolytic microbes and their enzymes for the development of industrial

sector. However, the prerequisite is to explore the natural habitat of Sri Lanka for these marvelous natural resources and institute a national policy for channeling those promising scientific findings to industrial scale applications.

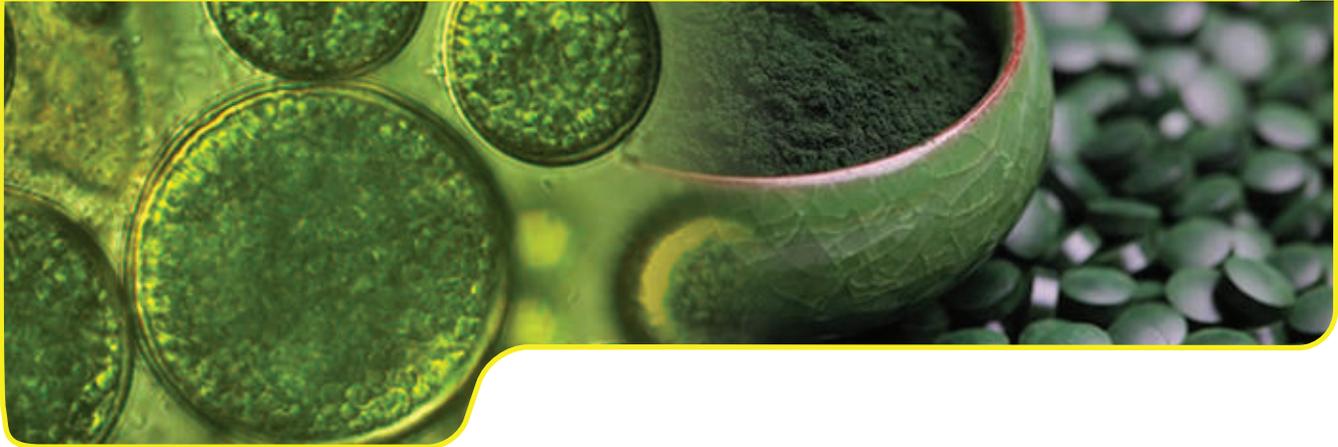


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## Microalgae, an underutilized microbial resource in Sri Lanka

Dr Thilini U. Ariyadasa



### 1. Introduction to microalgae

Microalgae are unicellular photosynthetic organisms found



**Figure 01: Diverse microalgae species (Source: Derwenskus and Holdmann, Microalgae – Underestimated All-Rounders, ChemViews Magazine, 2016)**

in aquatic environments which include freshwater bodies, marine environments and brackish water zones. Although microalgae are photosynthetic organisms, they differ from higher plants in not having roots, leaves or stems. Scientists have estimated that there are approximately 1 million species of microalgae on earth. As shown in Figure 01, morphology of microalgae varies drastically with

the species, making them a highly diverse group of microorganisms.

Microalgae are considered as primary producers of aquatic eco-systems, where they provide energy for all the higher trophic levels. In addition, microalgae generate oxygen during photosynthesis, attributing to nearly 50% of the total oxygen in the atmosphere. Moreover, being photosynthetic organisms, microalgae consume carbon dioxide in the atmosphere which contributes to alleviate the effect of global warming.

Over the past few decades, microalgae have emerged as a natural resource with great industrial potential due to

their ability of accumulating commercially valuable metabolites in high quantities. For instance, microalgae can synthesize carotenoids, lipids, carbohydrates and vitamins which can be employed in the production of pharmaceuticals, nutraceuticals, food/feed supplements, cosmetics or bioenergy. The rapid growth rates, higher photosynthetic efficiency, shorter harvesting time and higher biomass productivities (as compared to terrestrial plants) make microalgae a potential raw material for bio-based industries. Additionally, the cultivation conditions of microalgae can be easily manipulated according to the requirements. Furthermore, microalgae do not require arable land or potable water to facilitate their growth. Thus, cultivation of microalgae can be more sustainable as compared to land-based crops.

### 2. Microalgae-based bioproducts

Metabolites synthesized by microalgae can be extracted from the biomass and subsequently processed into diverse bioproducts.

## 2.1. Biofuels and Bioenergy

Several microalgae species are capable of synthesizing lipids up to 60-70% of their dry cell weight. The final lipid content is substantially affected by microalgal species as well as the culture conditions. Following the harvesting of biomass from cultures, lipid extraction is conducted from dried or wet microalgal biomass. Solvent extraction methods are the most widely used techniques, whereas solvent-free technologies are currently gaining interest due to lower potential of product contamination. The extracted lipids are then subjected to a process called transesterification where triglycerides are converted into alkyl esters in the presence

of a strong acid/base catalyst. However, microalgae-based biodiesel production is currently not economically feasible. In order to compete with petroleum-based fuels, the production cost of microalgae-based biodiesel should be decreased significantly.

In addition to the production of biodiesel, microalgae can also be employed for the production of bioethanol. This involves the hydrolysis of microalgal carbohydrates, which converts them into fermentable sugars such as glucose. Depending on the species, carbohydrate content of microalgae can reach up to 65% of dry cell weight, which is comparatively higher than lignocellulosic biomass. Enzymatic hydrolysis is preferred over chemical hydrolysis, due to

higher conversion yields, minimal by-product formation, mild operating conditions, and low energy input. However, further studies on economic feasibility of production are required prior to large-scale implementation.

Anaerobic digestion allows the generation of biogas from microalgal biomass. In this process, microalgal biomass is broken down into methane, water, and carbon dioxide by the activity of anaerobic bacteria. In general, anaerobic digestion is considered to be more economically feasible than biodiesel and bioethanol production, and showcases higher net energy ratios (i.e. ratio between energy output and energy input in biofuel production). Anaerobic digestion process is capable of

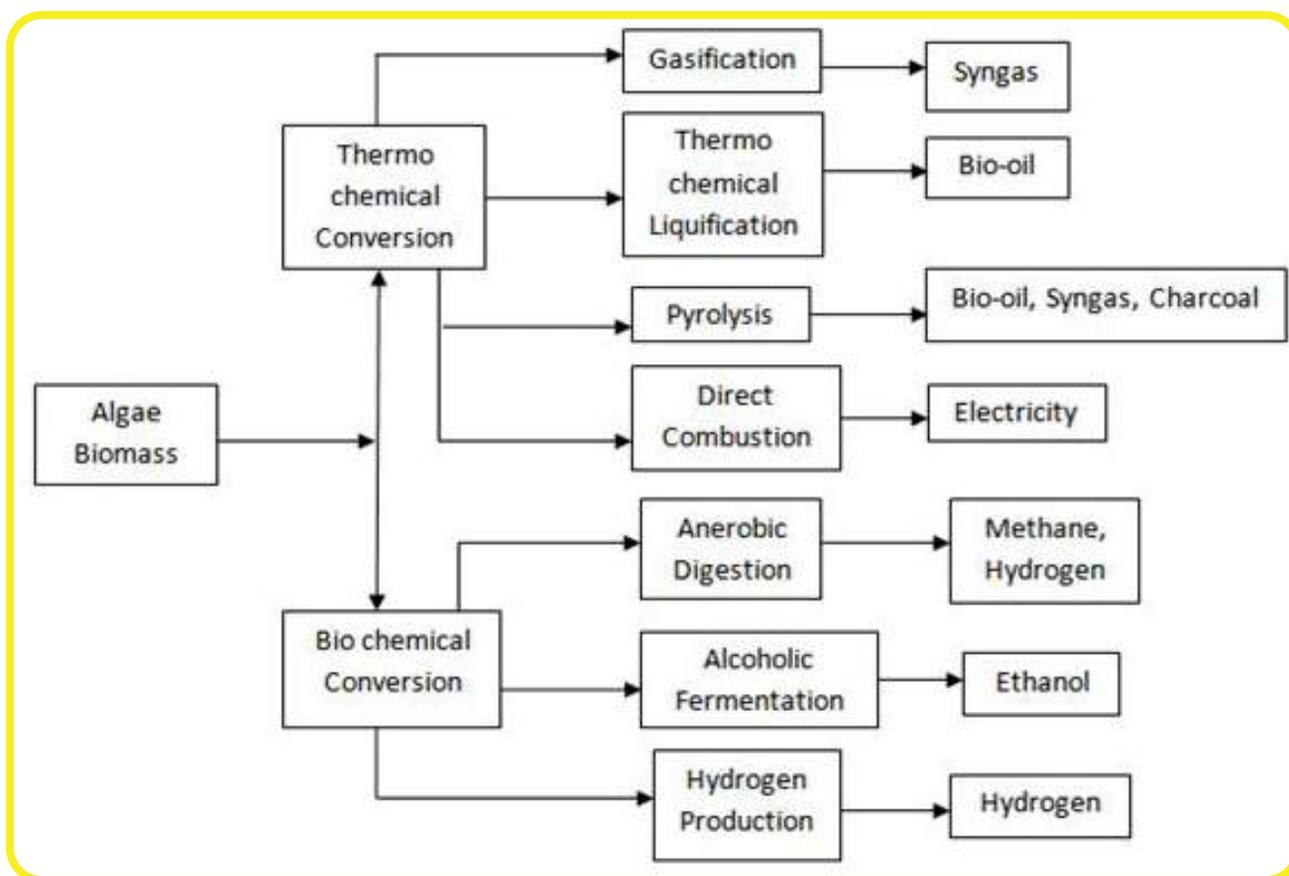


Figure 02: Biofuel and bioenergy from microalgae (Source: Chisti, Biodiesel from microalgae, *Biotechnology Advances* 25, 2007)



**Figure 03: Spirulina as food supplement (Source: Leech, 10 Health Benefits of Spirulina, 2018. <https://www.healthline.com/nutrition/10-proven-benefits-of-spirulina>)**

utilizing all the organic compounds in the microalgal biomass, whereas transesterification and fermentation utilize only lipids and carbohydrates respectively. Microalgal biomass can be co-digested with other substrates to enhance biogas yields.

Microalgal biomass can be subjected to thermochemical conversions which include pyrolysis, hydrothermal liquefaction and gasification. These processes generate biochar, biooil and syngas which can be utilized as energy sources. While existing facilities can be used for these processes, they involve elevated temperatures and pressures which can significantly increase the cost of energy. The different processes employed in the production of microalgae-based biofuel can be observed in Figure 02. Nonetheless, it is important to note that the most feasible conversion process for biofuel production from microalgae should be selected based on the biochemical composition of biomass, and a comprehensive techno-economic and life cycle analysis.

### 2.2. Pharmaceuticals, nutraceuticals and food/feed supplements

Microalgae can synthesize compounds such as carotenoids, proteins and vitamins which have high nutritional value. These compounds are either extracted from the microalgal biomass and encapsulated or consumed as whole

biomass. Carotenoids accumulated in microalgae with high nutritional value include astaxanthin, lutein, beta carotene and fucoxanthin. The biomass of microalgae such as *Spirulina platensis* (Figure 03) and *Chlorella vulgaris* are currently used as a human nutritional supplements due to their high protein content.

Considering the current state of technology, the use of microalgal biomass for pharmaceutical/nutraceutical applications is more economically feasible than the production of biofuels and bioenergy. This is due to the high market value of these compounds in comparison to the lower value of biofuels.

### 2.3. Biofertilizer

During their growth, microalgae can assimilate nitrogen, phosphorus, and carbon present in the growth media. Thus, the generated biomass is also rich in these elements, thereby making them a potential biofertilizer. By employing the microalgal biomass as biofertilizer, these elements are

introduced into the soil, which are subsequently absorbed by plants for their growth. In addition, microalgae biomass improves the texture of the soil. Furthermore, microalgae cultures can be added to soil as live inoculants instead of dried biomass. Live microalgae can secrete extracellular polymeric substances, produce plant growth hormones, fix atmospheric nitrogen and solubilize minerals in soil, which can substantially enhance plant growth.

### 2.4. Animal feed

Microalgae biomass is rich in essential fatty acids, proteins, vitamins and carbohydrates which are essential for the growth of living organisms. Hence, microalgae have been often used as animal feed and feed supplements to achieve enhanced quality of livestock as indicated by Figure 04. For instance, carotenoid-rich microalgae biomass has been used to improve the pigmentation of ornamental fish. In addition, use of carotenoid-rich microalgae in poultry feed has enhanced the nutritional value of hens' eggs and enhanced the color of egg yolk.

## 3. Microalgae in bioremediation

### 3.1. Carbon dioxide sequestration

Microalgae exhibit higher photosynthetic efficiencies than terrestrial plants, and hence showcase the ability to fix carbon dioxide from gas streams containing carbon dioxide concentrations far exceeding atmospheric levels (~0.04% v/v). Hence, numerous researchers have employed microalgae for

sequestration of carbon dioxide from industrial flue gas, which may contain the gas up to 3-30% v/v. Thus, the development of large-scale CO<sub>2</sub> sequestration processes would assist in the limitation of air pollution and global warming. Nonetheless, high temperature and potentially inhibitory constituents in flue gas can hinder microalgae growth, which necessitates further research and optimization of the growth conditions or pretreatment of flue gas.

### 3.2. Bioremediation of wastewater

Wastewater contains nitrogen, phosphorus and organic compounds which can be utilized by microalgae for biomass production. Thus, wastewater can be employed as an alternative growth media for microalgae. During their growth, microalgae assimilate these compounds, thereby removing them from wastewater. In addition, microalgae can remove heavy metals, dyes, and other toxic compounds from

wastewater through adsorption and biodegradation. Hence, numerous researchers have employed microalgae for wastewater bioremediation, and successfully achieved significant nutrient removal efficiencies. In addition to the effective treatment of wastewater, the integrated process is also advantageous in enhancing the sustainability and economics of microalgal biomass production. Accordingly, wastewater-based cultivation would result in the reduction of freshwater consumption and nutrient costs

incurred in the production of microalgae-based biofuels or bioproducts.

### 4. Microalgae as feedstock for biorefineries

One of the most significant challenges in the manufacture of microalgae-based bioproducts is the high costs in upstream and downstream processing steps. Because of this issue, it is currently not economical to produce low-value compounds such as biofuels. Nonetheless, the market value of microalgal metabolites used in pharmaceutical/

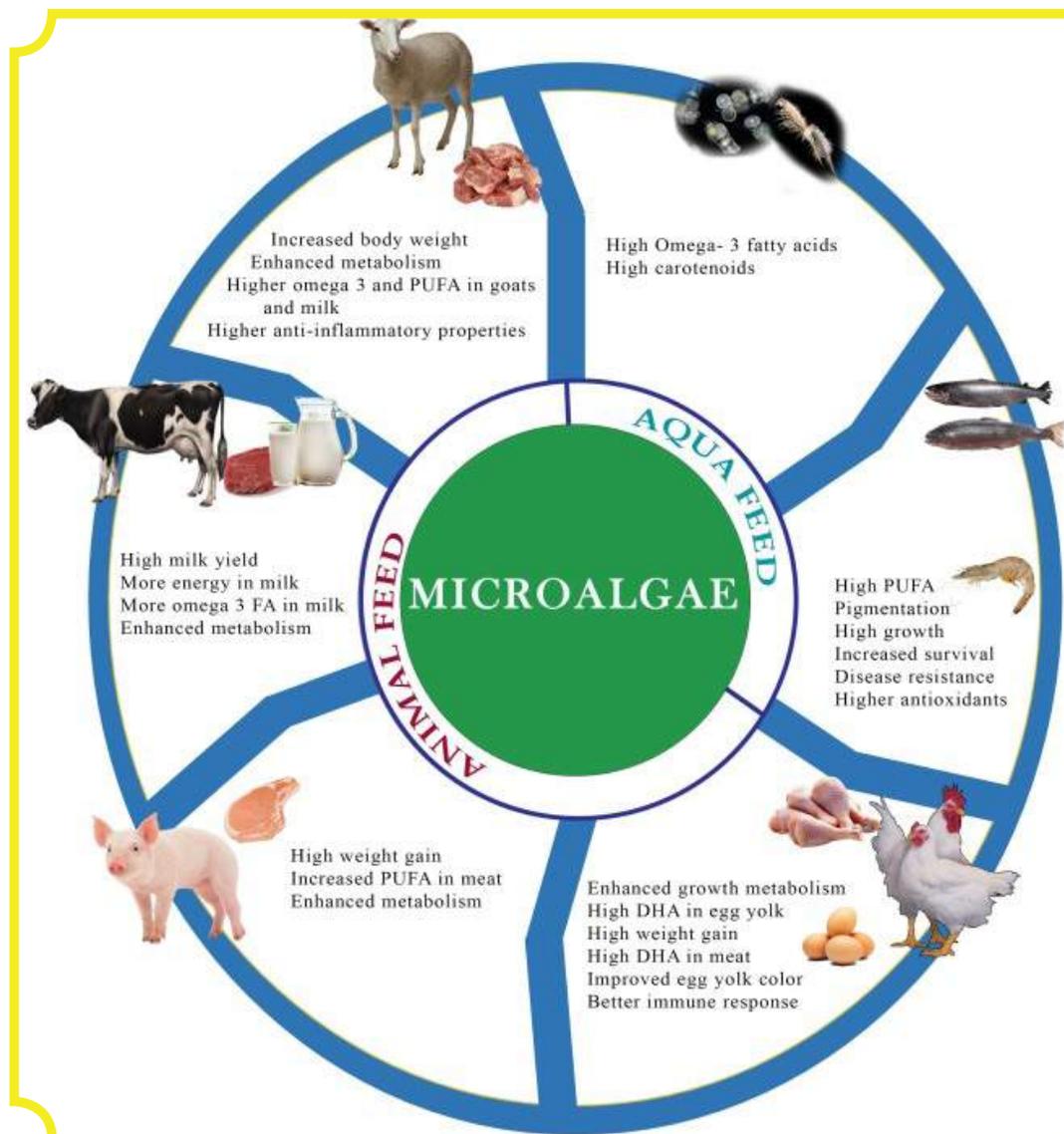


Figure 04: Microalgae as animal feed (Source: Dineshbabu et al., Microalgae–nutritious, sustainable aqua- and animal feed source, Journal of Functional Foods 62, 2019)

nutraceutical applications is markedly higher. Due to the lucrative nature of these metabolites, they are often termed as “high-value compounds”. Within recent years, the biorefinery concept has emerged as a potential method for holistic valorization of the microalgae biomass via the production of a diverse spectrum of bioproducts from a single biomass feedstock. In this scenario, the high-value compounds will be produced as primary products, whereas lower value compounds will be extracted from the residual biomass and converted to the desired bioproducts. Thus, the production cost attributed to lower value compounds would simply be the incremental costs incurred for processing of residual biomass, since bulk of the costs could be recovered from the revenue generated through the production of high-value compounds.

### 5. Microalgae-based research: the Sri Lankan scenario

Sri Lanka is an island surrounded by the ocean, which is rich in marine microalgal species. In addition, the inland surface waters of Sri Lanka include a large number of rivers and lakes. Despite the presence of different local microalgae species, Sri Lanka has barely tapped this valuable aquatic resource. Thus, the foundation for developing bio-based industries in Sri Lanka could be established by the exploitation of the diverse microalgae species within the country.

In order to identify microalgae species with biotechnological potential, sampling of inland and marine water bodies should be conducted, followed by

the isolation of species and subsequent identification. Thereafter, the isolated strains should be screened for their ability to synthesize metabolites of interest. Thereafter, extensive research should be performed to maximize the productivity of valuable compounds. This could be achieved through laboratory scale experiments for the identification of optimum culture parameters, which are highly dependent on

the field of microalgae because of the high biotechnological potential of these microbes. Currently, the microalgae research group at the Department of Chemical and Process Engineering, University of Moratuwa, are engaged in harnessing the underutilized national wealth of microalgae. The research group is currently working on isolation and identification of local microalgae species, screening of strains for target metabolite

production, optimization of culture conditions, microalgae-based bioremediation, microalgal biorefineries and pilot-scale microalgae cultivation systems. Their ultimate goal is to contribute to the establishment of microalgae-based industries within the country.



**Figure 05: Outdoor microalgae cultivation systems (Application of computational fluid dynamics for modeling and designing photobioreactors for microalgae production)**

the microalgal species. Following laboratory scale optimization, microalgae cultivation systems should be scaled up to increase the production of target metabolites. Being a country without any drastic seasonal variations and abundance of sunlight throughout the year, Sri Lanka could be an ideal location for the establishment of outdoor microalgae cultivation systems.

Hence, it is evident that Sri Lankan researchers should venture into



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## The key role of microbes in the energy grid as the master chemist of Earth

Dr Ama Damsara Jayawardana



The current issues in food and fuel prices have sounded an alarm to the world. Global energy supply and minimizing of climate changes are the two greatest challenges facing the 21<sup>st</sup> century society. The unseen populations of the microbial world, that include bacteria, yeasts, fungi, and archaea, can be helpful to address these challenges. Over the bygone diverse mutations, recombination events, and lateral gene transfer processes have transformed the microbes to access most of the theoretically possible oxidation-reduction reactions, as well as capture solar and other energy resources, and inhabit in a wide range of impossible and diverse environments. Some microbes are extremophiles, and exist beyond the current limits of the living environment. There are about  $10^{30}$  microbes estimated to be living in the Earth. Most of them are still unknown. These unknown microbes live on and in the human body. People encounter some microbes regularly in food, water and air. Others live in inhospitable places. Most of the microbes are beneficial, and their combined effort acts positively affecting numerous activities on

Earth. The microbes offer efficient ways to convert plants or other biomass, into liquid fuels, hydrogen, methane, electricity, or chemical feedstocks which are currently derived from fossil fuels.

### Microbes in biofuel production

For the purpose of energy production microbes can be sustainably utilized. For example, microbial fermentation of sugars and starch from food crops produces ethanol through the action of microbes such *Saccharomyces cerevisiae* (yeast). This ethanol can be blended with liquid transportation fuels. In Brazil, United States, and other European countries, ethanol-blended fuel is widely used (Figure 01). It is mainly a biofuel additive for gasoline. Ethanol is an alcohol used as a blending agent with gasoline, which increases octane and cuts down carbon monoxide and other smog causing emissions. Hence, biofuel protects the environment. However, it is expensive to convert edible plant materials into ethanol. Some ethical issues are also involved with this process. It has

been argued that people should not grow food stuff for fuel production purposes when people in some developing countries are craving for foods. There is global concern about countries like Brazil which may remove large sections of their rainforest to produce sugar cane in order to obtain ethanol. Deforestation of rainforests is a critical issue because these rainforests purify the atmosphere by removing a huge amount of carbon dioxide during photosynthesis. Because of this reason, production of biofuels from food stuffs such as sugar cane are unlikely, as long term solution to replace fossil fuels.

However, there is a large underutilized resource like cellulosic biomass from trees, grasses, and the nonedible parts of crops that could also serve as a feedstock. Scientists are investigating methods that can convert cellulose into ethanol. The ethanol produced from cellulose is the same as the ethanol created from edible plant parts. Usually, cellulose ethanol is generated from lignocellulose, which is a mixture of lignin, hemicelluloses and cellulose. These three materials are the main

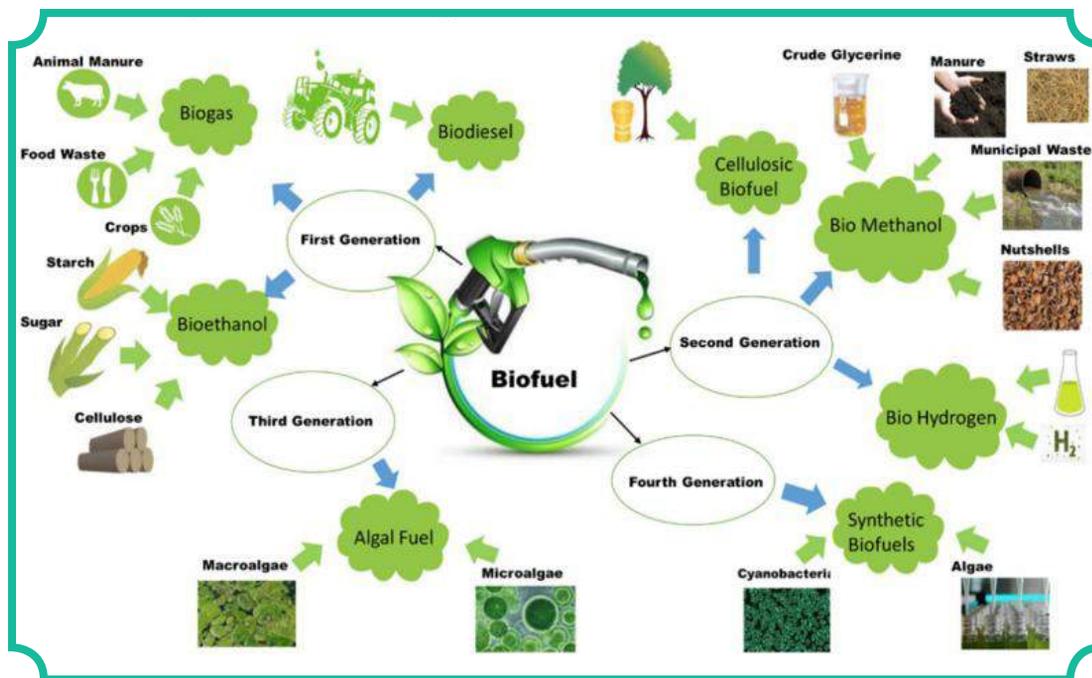


Figure 01: Biofuel production

live inside root nodules of plants (*Rhizobium bacteria*). They also inhabit stems, root surfaces and leaf surfaces. The individual microbial activities provide plants with nitrogen which decreases the need for fertilizers. Their individual activities also help plants to access phosphorus, recycle nutrients, improve soil structure and protect plants from various diseases. These

microbial plant

associations can improve crop production on marginal lands. Ultimately, these benefit both food and biofuel production. In addition to that, successful microbial plant associations may also help to abate current food versus fuel debate, and thereby lessen the environmental foot print of agriculture.

### Microbes in hydrogen and methane production

The microorganisms that produce methane as a metabolic by-product in low oxygen conditions are known as methanogens. They are prokaryotic organisms that belong to the domain of Archaea. Some of the well-known examples are *Methanosarcina barkeri*, *Methanobrevibacter smithii*, *Methanosarcina acetivorans*, *Methanococcus maripaludis*, *Methanocaldococcus jannaschii*, etc. They are very common in wetland areas in the world. These prokaryotic organisms are responsible for swamp gas

components of the plant cell wall. The lignocellulose is the part that is undigested by human beings and most animals after consumption of plant based food sources.

Lignocellulose is also a part of non food stuff, such as stalks, saw dust and wood chip. There is therefore a huge amount of non edible plant waste that can be recycled. Through long term research scientists have already found some microbes that produce the group of enzymes called cellulase that can convert cellulose to fermentable sugars. There are three main members in this cellulase family; endoglucanase, exoglucanase and  $\beta$  glucosidase, which convert lignocellulose into fermentable sugar. These fermentable sugars subsequently produce ethanol by the action of yeast. Archaeans such as *Sulfolobus solfataricus* that lives in volcanic pools near Mount Vesuvius in Italy, and wood digester fungus *Triderma reesei*, which is found in all soil types, are some of the well-known examples of microbes

producing cellulase enzyme. With genetic modifications to these microbes, it is possible to improve the performance of these microbes to produce more cellulase enzyme. Furthermore, a company in Canada has already genetically modified a fungus that can convert straw into glucose. A staggering amount of straw fiber is converted into sugar through this fungus. The glucose is then fermented with yeast to produce biofuel ethanol.

Research programs are underway to use metagenomic, synthetic, and other approaches to identify microbes, enzymes, or microbial communities that release sugars from cellulose. These ultimately convert sugars into ethanol or other fuels. Therefore, the microbial-plant ecofriendly relationships can improve the sustainability of biofuel production in the world.

The sustainability of biofuel production can be improved by the microbial plant relationship in different ways. Typically, microbes

(methane and other gases). These methanogens break down organic compounds to produce hydrogen (H<sub>2</sub>), carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>). Normally, this breaking down process occurs in the digestive tract of domesticated

The hydrogen that is produced while converting organic matter to methane, is also very useful. Hydrogen can be used to generate electricity, power and heat. Currently, hydrogen is commonly used for refining of petroleum and

bioelectrochemical system. One of the examples for bioelectrochemical system is the microbial fuel cell (MFC) (Figure 03). The microbial fuel cell has one anode chamber (negative electrode) and one cathode chamber (positive

electrode). A microbial fuel cell functions very similar to a normal battery. In microbial fuel cells, microbes decompose organic and inorganic matter in an anode chamber which results in electrons. Later, these electrons flow from anode chamber to cathode chamber through an external circuit made up of conductive material. The conductive material used is usually copper based wires. Ultimately, this biochemical reaction produces electricity. Many companies in the world are already involved in MFC technology. Companies that are applying MFC technology in a

commercial scale include EcoVolt by Cambrian Innovation, and VIVA MFC by MICROrganic Technologies in United States, Prongineer in Canada and Plant-e in Netherlands. These companies integrate plant microbial fuel cells (PMFCs) in their MFC technology. The most researched electrochemically active bacteria (EAB) in microbial fuel cell technology are *Geobacter sulfurreducens* str.PCA and *Schewanella oneidensis* str.MR-1.

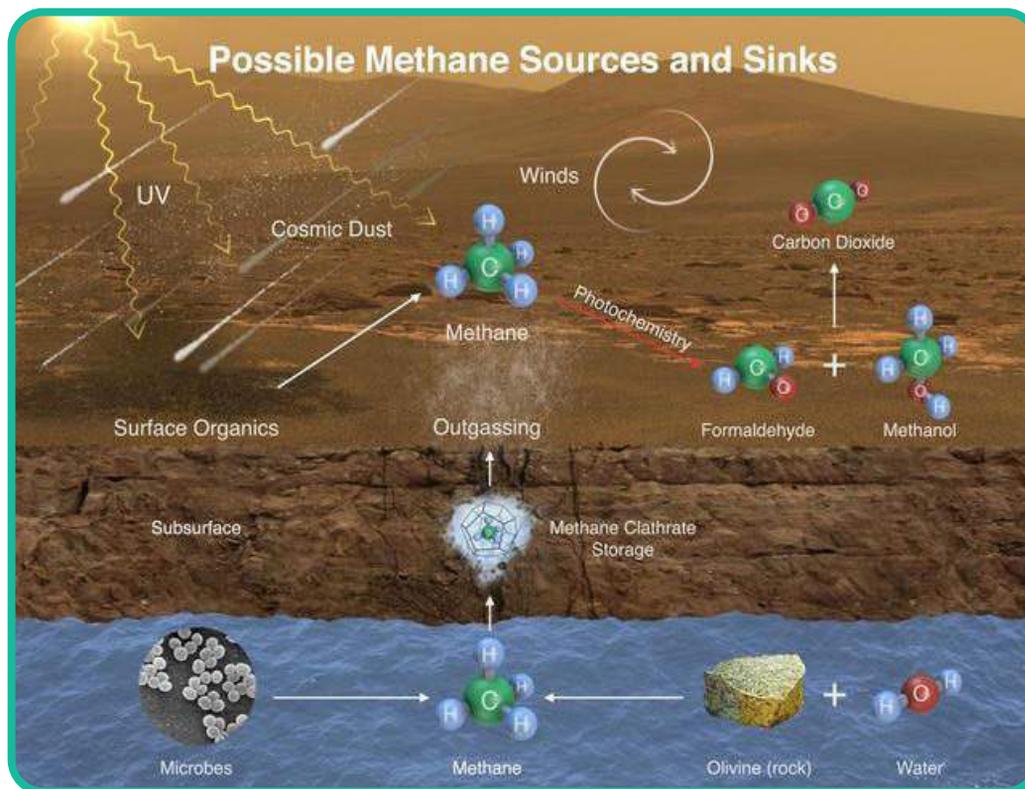
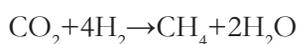


Figure 02: Methane production

and wild ruminants other than in the wetlands and rice patties. Methanogens are also responsible for the generated methane content of belching in ruminants, and flatulence in humans. Methane is a colourless, odourless and inflammable gas (Figure 02). Methane is primarily used as a fuel to generate heat and light. Other than that methane is also used to manufacture organic chemicals. The reaction for reduction of carbon dioxide into methane in the presence of hydrogen is as follows;



fertilizer production. Furthermore, methanogenic archaea play a vital role in anaerobic wastewater treatment.

### Microbes in electricity production

It is possible to generate electricity from microorganisms. This is an alternative approach to the usual electric power from water, wind, solar and steam. Scientists have been studying the ability of microorganisms such as bacteria to generate electricity. For this particular purpose scientists are using a special system called

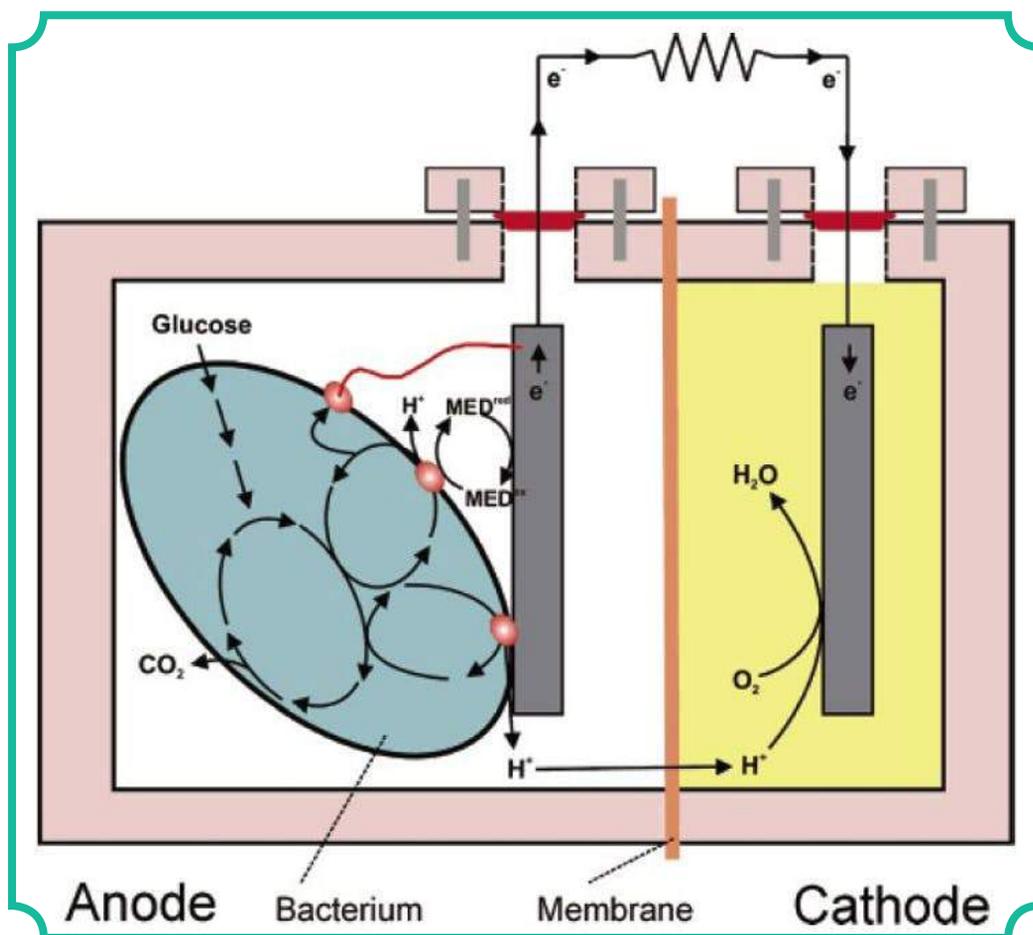


Figure 03: Microbial fuel cell- (MFC)

### Microbes in chemical feedstock production

In chemistry a chemical feedstock is a substance that is used to support a large scale chemical reaction. For example, petroleum is the chemical feedstock for gasoline and other chemicals including methane, propylene and butane. But petroleum based industries are criticized due to environmental pollution and their high cost of production.

Domestic biofuels are really good alternatives for petroleum based transportation. Biofuels are typically produced from plant materials like oils, sugars and biomass. The plant matter is derived from photosynthesis. Photosynthesis

is the process of converting solar energy into chemical energy in plants. Photosynthesis however is not an efficient way to transfer energy from the sun to a plant and then to biofuel. So, incorporating electrofuel that bypasses the photosynthesis process by using microbes, can directly use energy from electricity and chemical compounds to produce liquid fuels.

Recently, Massachusetts Institute of Technology (MIT) in United States developed a new technique that uses carbon dioxide (CO<sub>2</sub>) and hydrogen (H<sub>2</sub>) generated from electricity to produce natural oils which can later be improved to hydrocarbon fuels. For this purpose MIT has developed a 2-stage biofuel production system.

In the first stage CO<sub>2</sub> and H<sub>2</sub> are fed to a microorganism that is capable of converting these feedstocks to a 2-carbon compound called acetate. Then in the second stage the acetate is delivered to another microorganism that can use acetate to produce natural oils. Later, the oil can be removed from reactor tank and chemically converted to various hydrocarbons. The electricity for this process can be supplied from the combustion of municipal waste. Combustion of municipal waste would also produce the required CO<sub>2</sub> and hence enhance the efficiency of biofuel production system. For the above

purpose acetogenic and methanogenic bacteria can be used (Figure 04). Further, chemical feedstocks such as 1,4-butanediol and malic acids are produced by using microorganisms such as *Escherichia coli*, *Aspergillus* and *Ustilago trichophora*. 1,4-butanediol is produced through *Escherichia coli* bacteria which is used to make floor stripper, paint thinner and other solvent products industrially. While, malic acid is produced by using fungi such as *Aspergillus* and *Ustilago trichophora*, Malic acid is used in medicine, in food industry as a flavoring agent, and to resolve 1-phenylethylamine.

### Microbes in mitigating climate changes

Plants and autotrophic microbes

can also be helpful in mitigating climate change, because they sequester atmospheric carbon dioxide at a higher rate. This depends on the subsequent oxidation of soil and plant carbon by microbes. The potential way to mitigate climate change is through reduction of green house gases. The green house gases are increasing due to human activities and natural factors. For example, green house gases increase through combustion of coal, oil, fossil fuels, decay of plant matter and burning of biomass. At the moment climate change and the global warming are the major

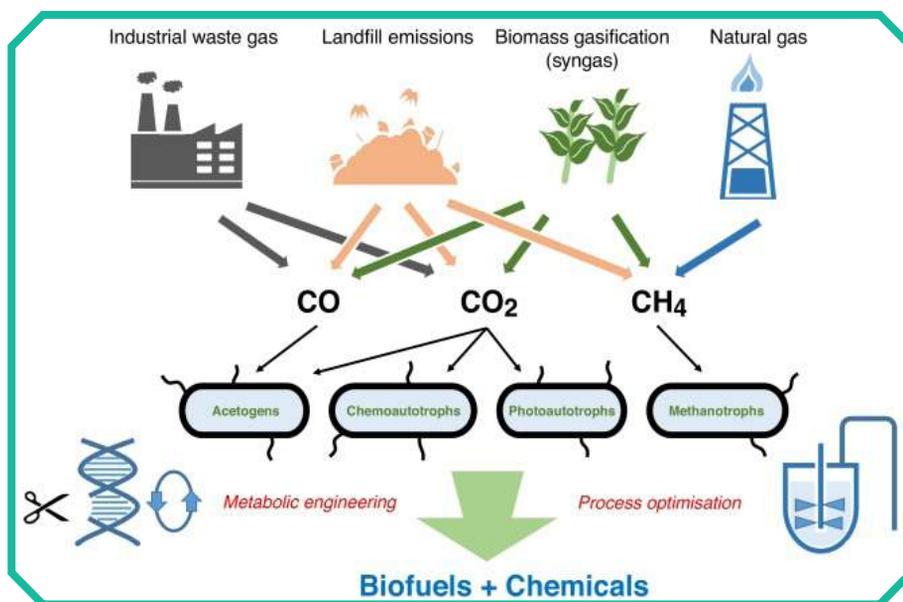


Figure 04: Chemical feedstocks

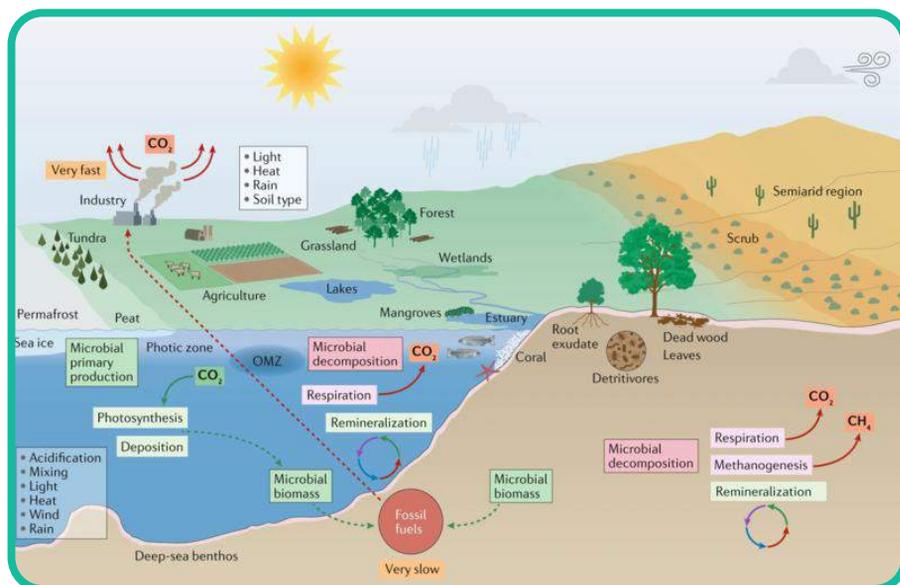


Figure 05: Microbes in mitigating climate changes

problems in the world. Climate change and the global warming can cause more frequent severe weather conditions, higher death rates, dirtier air, higher wildlife extinction, acidic ocean, higher sea level, etc. In order to compromise climate changes there are several methods. One of the methods is the use of microorganisms and other biological components that can mitigate climate changes. The autotrophic microbes reduce

green house gas through nutrient recycling processes. The marine phytoplankton is a good example of an autotrophic microbe that is involved in global photosynthetic CO<sub>2</sub> fixation (Figure 05). Furthermore, bacteria belong to families such as *Comamonadaceae* and *Sphingomonadaceae*, which can also be used to reduce green house gas. A concerted effort in co-ordinated research would be crucial in order to get the maximum use of advantages referred to above

microbes acting as master chemists can help the global population to face the challenges of energy, encounter pollution, and ensure adequacy of food. All in all, the rich genetic blueprints of microbes will provide new energy sources while improving the health of the biosphere.



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# QUESTIONS And Answers

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**What have you learnt from the Vidurava 2021 July - September Q<sub>3</sub> Issue? Scan your own memory!**

## **1] Living microbes: An invaluable global wealth**

True or False?

- 1.The discovery of microorganisms by Anton Van Leeuwenhoek brought about a revolution in science.
- 2.The improvement and application of biological N<sub>2</sub> fixation in crop production gave a special impetus to microorganisms.
- 3.It has been reported that soil microorganisms frequently associate as individuals with roots of higher plants.
- 4.On the positive side there are possibilities to develop genetically modified more virulent plant and animal pathogens including those against human beings.
- 5.The oxygenation of the atmosphere also triggered off the development of aerobic process of respiration.

## **2] Use of microbes in next generation biofertilizers**

True or False?

1. The emerging omics technologies which include next generation sequencing, microarrays and other chip based approaches have revolutionized the field of agriculture.

2. A rhizosphere metagenomic study conducted in oil-contaminated soil has revealed the significant presence of hydrocarbon degrading microbes.

3. Conventionally, research on extremophiles have not been dependant on culture-based techniques.

4. Although targeted sequence metagenomics identify the potential microbes that could be used as inoculants for biofertilizers, it can only confirm the taxa involved.

5. The workflow used in metaproteomic studies does not have a direct impact on the outcomes.

## **3] Phosphorous solubilizing microbes: A promising approach to minimise triple super phosphate usage in rice cultivation**

True or False?

- 1.Triple Super Phosphate (TSP) is widely used as a synthetic phosphate fertilizer in Sri Lanka.
- 2.Phosphate rock undergoes several acidulation processes in the TSP manufacturing process.
- 3.Eppawala Rock Phosphate (ERP) is a costly, phosphate-rich source used as a P fertilizer.
- 4.The Phosphorous Solubilizing Microbes (PSMs) are a group of heterotrophic microorganisms capable of solubilising the inorganic forms of P by excreting organic acids.
- 5.Applying Biofilmed Biofertilizers (BFBFs) in rice cultivation has significantly increased chemical fertilizer usage by about 50 %.

## **4] Cellulolytic microbes: Potential living resources for industrial applications in Sri Lanka**

True or False?

- 1.Initially microbes were mostly known as accelerators of domestic food processing practices.
- 2.Microbes were recognized as dispensable biological resources in more advanced biotechnological applications.

### 1] Living microbes: An invaluable global wealth

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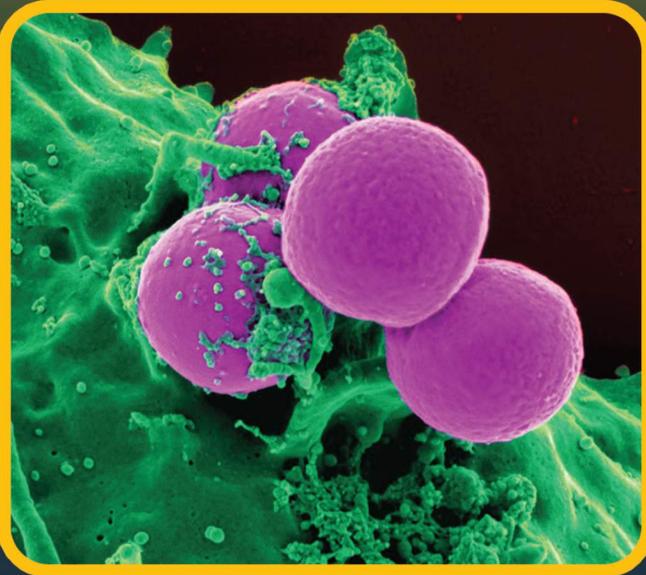
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01) 1. True, 2. True, 3. False, 4. False, 5. True	02) 1. True, 2. True, 3. False, 4. True, 5. False	03) 1. True, 2. True, 3. False, 4. True, 5. False	04) 1. True, 2. False, 3. False, 4. True, 5. True	05) 1. True, 2. True, 3. False, 4. True, 5. False	06) 1. True, 2. True, 3. False, 4. True, 5. False
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Answers



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