# Factories, microbes and biotechnology

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In the last fortnight, microcellular organisms have found themselves at the centre of yet another strategic piece of scientific engineering dealing with lights and optics. In a parallel approach, a team of scientists led by Deepak from the Indian Institute of Technology, Kanpur (IIT-K), have utilized yeast cultures as templates for constructing micro lenses which can enhance the performance of organic Light Emitting Diodes (LEDs)[1].

Although conventional LEDs produce intense bright light, they have to be fitted with external contraptions to create a diffused lighting effect. This property is inherent in organic LEDs right from their conception. However, the intensity of light produced is not at par with the conventional, commercial variants. Microlenses can solve this problem by minimizing the loss of photons, but synthesizing such structures present numerous hurdles.

A new method developed by scientists in IIT-K can solve this problem. To make these microlenses, the researchers mixed Bakers' yeast – *Saccharomyces cerevisiae* – with an appropriate medium and dispensed it onto a basement membrane using an ink-jet printer. The method yields two-dimensional patterned prints which turn into 3D templates post-incubation due to the growth of yeast. These could then be utilized for casting microlenses using appropriate synthetic materials.

This technique has largely overcome the problem of cost intensive and tedious processes using material based inks which offers low viscosities that makes it nearly impossible to get the desired aspect ratio. Moreover, the perils of using such material are also overcome by the new technique.

There is no doubt that microbes are shaping our world in ways yet unfathomable to human kind. Amidst studies that report their effect on our mood, behaviour, identity, immunity and obesity, there are studies that reflect upon the scientific progress made by humans and how microbial systems are assisting in writing the tale of our scientific progress. One way this could be achieved is through biotechnology.

Interestingly, humans have been using simple biotechnological approaches since even before the term biotechnology was coined[2]. Microbial cultures were used by several civilizations for making wine, cheese and yogurt[3]. The discovery of the first microscope enabled the study of cells and unicellular organisms which paved the way for modern biotechnology. A landmark achievement was the discovery of penicillin by Alexander Fleming which to this day keeps infections at bay and forms the basis of modern medicine[4]. About 20 years after the discovery of penicillin, microbes were used to produce huge quantities of penicillin for the general populace. This was the first time that microbes were used at such a large scale for production of a commercially important substance[5]. It further propelled the study of the bacterium and its modus operandi.

Scientists found that since bacteria inhabit every inch of our planet they are often equipped with unique machineries that are capable of synthesizing novel chemicals which boast of diverse applications. Scientists have identified and extracted large classes of novel compounds. One such example is surfactants. They are unique in the sense that the compounds contain both water soluble and fat-soluble parts. When added to a water-oil formulation, they can help in the mixing of constituent fluids which are otherwise immiscible. This finds use in a lot of industrial processes, particularly the food industry where it is used to create a variety of textures. The creamy flavour of ice creams, chocolates and various dips are all a result of addition of surfactants or – what they are commonly known as – emulsifiers, to the mixture. Presently, most food products contain synthetic emulsifiers. Recently, Negi and her team hailing from the Motilal Nehru National Institute of Technology, Allahabad, have extracted an emulsifier from a previously unstudied strain – *Bacillus* sp. MTCC5877 (ref. 6). After isolation, the compound was characterized and compared with commercially used emulsifiers. What the team next found is amazing. The chemical could not only reduce the tension of water droplet from 70 to 30 mN/m but was also found to possess strong antibiotic property. And what is more, it serves to reduce heavy metals from plant tissue. As such, it boasts of multifarious applications that have a huge potential in industry. The emulsifier could offer protection from food spoilage and could also reduce the use of synthetic additives in food products that have recently become the bane of our lives.

But this does not even begin to illustrate the full spectrum of opportunities that microbes could provide us. In fact, by borrowing simple bacterial machinery, CRISPR/cas, scientists have brought about a paradigm shift in genetic engineering. Now, using this molecular machinery, it is possible to integrate any gene in any genome at any location.

Everyday bacterial cells undergo mutations and acquire new properties which can be optimized in astounding ways. Microbial fuel cell is a typical example. As the name suggests, such cells run on the energy of electrogenic bacteria capable of releasing electrons which can be accepted by terminal electron acceptors at cathode to generate electricity.

Presently, single bacterial species and mixed bacterial cultures are being tested in microbial fuel cells for commercial purposes. In theory, the use of mixed culture offers wider range of substrate utilization, high substrate consumption and high power density. But in practice, mixed cultures have consistently been less effective in terms of energy generation. Studies have revealed that competition between non-electrogenic bacteria and electrogenic microbes compromise the efficiency of the system.

Mohan from the Indian Institute of Chemical Technology, Hyderabad and his team have solved this problem by pre-treatment of such cultures[7]. They used iodopropane (specific methanogen inhibitor) and heat shock treatment (non-specific inhibitor) for enriching the electrogenic population in microbial fuel cells. The cultures were either treated with iodopropane or subjected to heat shock at 80 degrees and left to culture for 24 hours. After the incubation period, the electrogenic parameters were analysed.

Current and voltage of these systems were continuously monitored and additionally, biochemical parameters like chemical oxygen demand (COD), volatile fatty acids (VFAs) and pH of the system were determined. COD removal efficiency of a system is directly proportional to degradation capacity whereas coulombic efficiency (CE) is an indicator of amount of electric current that could be produced by the system.

Iodopropane-treated cultures exhibit the highest coulombic efficiency of 6.188% with 52% of COD removal as compared to heat treated cultures (4.75% of CE with COD removal of 51%). Both techniques showed better results than the untreated culture that could produce only 2.89% of CE.

The results of the study clearly illustrate the benefits of bacterial enrichment which supports the initial hypothesis that both iodopropane and heat-shock pretreatment can eliminate competitors. Iodopropane-treated MFC also demonstrated higher power output compared to other MFCs. Scientists reason that due to the suppression of methanogenic pathways, chemical inhibitors do not affect the growth of other species as opposed to heat treatment. The technique also sustains stable power production without compromising the integrity of the system.

In the wake of problems posed by the indiscriminate use of non-renewable energy sources and its associated environmental hazards, the use of microbial fuel cells can provide a means for sustainable energy production. What is more enticing is the possibility that these cells could be integrated with other biological systems like wastewater treatment plants to make the process more viable.

Shantanu Roy, a biotechnologist from the Indian Institute of Technology, Kharagpur has published an article highlighting the myriad ways in which electroactive bacteria could be utilized for value added product synthesis[8]. A particularly interesting scheme involves coupling the electron transport of electrogenic bacteria to carbon synthesis. The membranes of several bacteria contain elements of the electron transport chain like cytochrome c which can donate electrons to the external environment. This exchange of electrons between microbes and electrodes can overcome redox limitations that restrict the synthesis of high value multi-carbon compounds. By utilizing this strategy, waste streams from different industries could be used as feedstock for carbon fixation and the intermediates thus produced can be connected to other metabolic pathways. This offers immense opportunities for metabolic engineering. The microbial enzymes could thus be employed to convert biomass-derived carbon sources into many unique molecules.

Due to their simple machinery, ease of culture and small doubling times, microbes can be used as miniature factories for the production of therapeutic proteins, medicines and even other unique classes of chemicals. Such alternate biosynthetic approaches may provide sustainable power and hold the key to survival of our civilization in the near future.

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