How to be the perfect host

Professor Yusuf Chisti is a world expert in the production and processing of biotechnology products.

In a milky, nutrient-rich broth in a glass bioreactor at AgResearch myriads of bacteria are dividing and growing.

When harvest time comes, a few drops of a chemical inducer will be added to the solution, a gene will switch to 'on', and small bodies in the bacteria called plasmids will begin industriously fabricating a protein to order. So much protein will accumulate that the cells will create so-called inclusion bodies to contain it.

The protein is an antigen, a substance that stimulates the production of antibodies in animals injected with it. This antigen is normally associated with the eggs of a hydatid tapeworm. The bacterium is a modified version of the rod-shaped *Escherichia coli* bacteria which live in animal intestines, including our own. It has no use for the protein.

But a sheep vaccinated with the protein will develop an immunity to hydatidosis, a disease, which, while officially eradicated from New Zealand in 1999, continues to flourish in other parts of the world, infecting and sometimes killing human hosts.

In another bioreactor a genetically engineered yeast is producing follicle-stimulating hormone (FSH), which can be used to induce 'superovulation' – the development of more than the usual number of mature eggs – in humans and other animals. This is ovine FSH – the FSH produced in sheep.

In a third bioreactor, ovine FSH is again the product, but this time the cell line is a gene-recombinant CHO (Chinese hamster ovary) cell favoured by researchers.

Masterate student Daniel Manderson's interest in this is not in the technology that has cut and spliced the genes from one organism into another. Supervised by Massey's Professor Yusuf Chisti and Dr Robert Dempster of AgResearch, he is setting out to create the perfect environment for these very different cell lines to grow and thrive. Inside each vat, nutrients and oxygen must be delivered in precise quantities, the optimum temperature and pH maintained, and wastes removed.

These bacterial, yeast and mammalian cells are picky about their living conditions. Manderson's task is to make AgResearch the perfect host.

For millennia humanity has turned micro-organisms and their natural by-products to its advantage. Yeasts have converted sugars in grape juice to the carbon dioxide and alcohol in wine, and bacteria have cured cheese since well before anyone had an inkling that there were such things as yeasts or bacteria. In the last few hundred years smallpox vaccines have been made from viruses and, in the last 60, penicillin has been manufactured by culturing moulds. (Alexander Fleming, who discovered penicillin, is famed; the fermentation expertise in the US that made penicillin a cheap wonder drug is less well known.) This is all part of the biotechnology industry's lineage.

But the biotechnology phenomenon – biotechnology as stock market darling and harbinger of a brave new world – is recent.

All life as we know it – be it bacterium, beech tree or banker – shares a common origin and a common code, written in DNA's iconic double helix of base pairs between two strands of nucleic acid. DNA, to oversimplify, does two things: it produces copies of itself, and it produces RNA, which makes proteins.

The structure of DNA was postulated in 1953, but it took time to tease out the implications and longer still for there to be practical applications. DNA was effectively 'read only'. Then in 1972 Paul Berg published a paper announcing the cutting and pasting of DNA between *E. coli* and a virus. His techniques, it was apparent, would have wide application. If you could transplant DNA you could make proteins to order.

One of the first breakthrough uses of gene recombinant technology was in producing insulin used in the treatment of diabetes. Until then the insulin was sourced from pigs, cattle and human cadavers. Insulin is produced in the pancreas, and pancreas cells are difficult to culture. By contrast *E. coli* – the rod-shaped intestinal bacterium we met earlier on – is a breeze. In 1979 recombinant *E. coli* was persuaded to produce the components of insulin. In 1982, synthesised insulin produced by genetically altered bacteria was approved for use with insulin-dependent patients.

The air was heady with possibilities.

Professor Yusuf Chisti is an energetic, olive-complexioned man with a transatlantic accent touched with a dash of somewhere else. This proves to be Nigeria, where the Pakistan-born Chisti spent his life between the ages of nine and 20, when he completed his first degree, in chemistry at the University of Jos.

Master's student Daniel Manderson examines one of the cell cultures being grown at AgResearch.
(Photos courtesy of AgResearch)

Gene recombinant *E. coli* bacteria showing inclusion bodies – the darker regions – where foreign proteins are isolated. The protein being produced here is used in a vaccine that can protect sheep against hydatidosis, a disease eradicated in New Zealand but still prevalent in many other parts of the world. AgResearch, which developed the vaccine in collaboration with Melbourne University, has commercial partners in China and Argentina.

Industry training programme to be piloted

A $400,000 pilot programme to develop industry training within the biotechnology sector has been announced by Pete Hodgson, Minister of Research, Science and Technology. The programme will be led by Professor Chisti.

The pilot programme will run high-level, enterprise-specific modular workshops for biotechnology firms. The content of the workshops – expected to concentrate on the areas of biotechnology science, processing, regulatory compliance and business – will be developed in consultation with industry

"We have already proven that New Zealanders are world beaters in biotechnology. This initiative will help consolidate and grow that position," said the Minister.

The project will also call on Professors Ian Maddox and Richard Archer of the Institute of Technology and Engineering; Professor Barry Scott and Associate Professor Bernd Rehm of the Institute of Molecular Biosciences; Associate Professor Alan Murray of the Institute of Veterinary, Animal and Biomedical Sciences; and Dr Gavin Clark of Research Services.

The pilot forms part of the Government's Growth and Innovation Framework, which is funded through the Tertiary Education Commission's contestable funding initiative and is intended to promote closer collaborations between tertiary education organisations and industry.

Professor Chisti's choice of major was driven by pragmatism: Jos did not teach engineering and Professor Chisti did not think of himself as strong in mathematics. His choice for his second degree was driven by ambition: "I wanted to get into something 'hot'," he says.

So in 1979 – that same year insulin was first produced – Professor Chisti headed to University College, London, to begin a masterate in biochemical engineering.

After completing his masterate, and two years of teaching back in Nigeria, Professor Chisti followed with a PhD in biochemical engineering at Waterloo University in Ontario, Canada. His thesis topic was airlift bioreactors. Professor Chisti would go on to literally write the book on bioreactors: *Airlift Bioreactors* by Yusuf Chisti was published in 1989 and has been cited in the academic press on average once a month ever since.

Above Professor Chisti's desk is a strange calendar. It features page-on-page of bioreactors: gleaming stainless steel tanks with tubes, valves and gauges. The lighting is an eerie blue; the settings are aseptically clean and ordered. At least one of the pictures has classical art works set about the stainless steel. Of people, there are none.

Bioreactors are fundamental to the biotech industry. The stainless steel bioreactors used in the production of human therapeutic proteins will usually run to a volume of between 2,000 and 10,000 litres, though some reach volumes of 100,000 litres. The most common are stirred-tank bioreactors, with the stirring being provided by impellers that resemble boat propellers. But with this method of mixing comes shear stress. "Just as when you have a storm, the wind can uproot trees or lift roofs, the forces in a liquid can tear apart suspended cells," explains Professor Chisti.

In an airlift bioreactor the circulation of fluid is driven by the release of gas into the lower portion of the bioreactor and a draft tube helps ensure thorough mixing without creating disruptively strong currents.

Recently Professor Chisti has been experimenting with the use of carefully calculated levels of ultrasound, which has been shown to improve mixing and lift cell productivity.

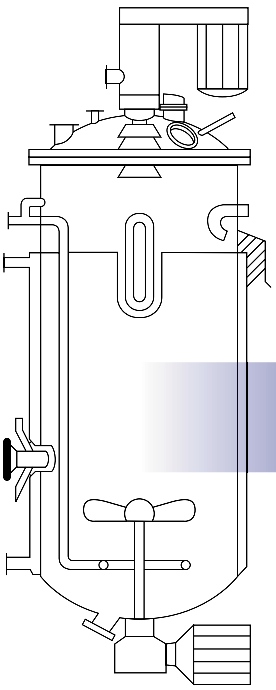
On finishing his PhD and completing a one-year postdoctoral fellowship at Waterloo, Professor Chisti joined a wealthy biotech start-up, Chembiomed, in Edmonton. Here, as one of their 100 or so employees, Chisti designed and built the first GMP (Good Manufacturing Practice) compliant monoclonal antibody production facility in Canada.

Monoclonal antibodies are used in diagnostic tests, or assays. The diagnostic test for HIV AIDS uses monoclonal antibodies, as does the over-the-counter home pregnancy testing kit. Professor Chisti's facility produced the monoclonals used in testing for blood types.

The 'monoclonal' part simply refers to the antibodies all coming from clones of a single cell.

Although human and animal cells produce antibodies, there is a problem with using these cells for commercial production: after dividing a number of times, the cells die. To get around this, the practice is to take the cell that produces the antibody and fuse it with a cultivated tumour cell, producing an immortal cell line called a hybridoma. Usually these are mouse cells.

Hybridomas, being sensitive to shear stress, are best cultured in airlift bioreactors.



The schematic for a stirred-tank bioreactor. An airlift bioreactor uses the release of gas rather than an impeller to provide mixing.

In 1991 Professor Chisti returned to Waterloo as an adjunct professor. His next move was to the south of Spain in 1997.

"I had a postdoctoral visitor working with me when I was in Canada, and he became a professor at this Spanish university, and invited me to come over. So I spent three-and-a-half years in Spain in a pleasant town called Almería right on the Mediterranean coast across from Africa," says Professor Chisti.

At Almería Professor Chisti taught postgraduate students and became involved in the research administration relating to projects sponsored by the European Union. He also developed an enduring interest in microalgae.

Canada had been cold. In Waterloo the average temperature in January is minus 8 degrees centrigrade; in Edmonton it can hit minus 40.

By contrast, Almería has more cloudless days than any other region in Spain, and its average temperature is 18. It is a setting that lends itself to the filming of westerns, holiday houses for jaded Brits, and, with its warm temperatures and seawater, the growing of microalgae.

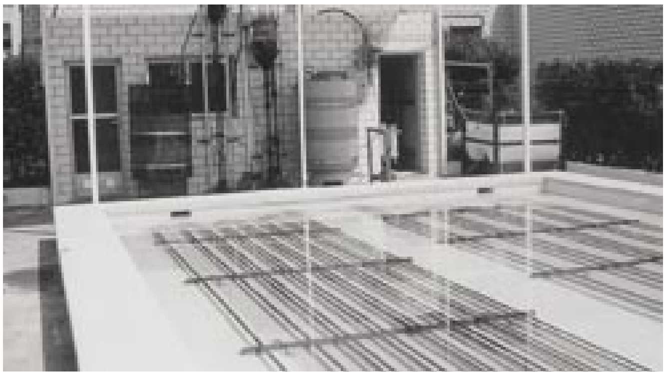
Currently microalgae, which make up 60 percent of the Earth's primary productivity, are scarcely used in commercial processes. This is not to say they have no uses at all. Spirulina, a cyanobacterium, is sold in the form of drinks and health food supplements. Chlorella, a microalga, is another health food supplement. In Western Australia a microalga is farmed for the production of beta carotene, a substance found in fruit and vegetables. Beta carotene is used as a food colourant, dietary supplement and an ingredient in cosmetics. In places like Hawaii and California a microalga is grown to be harvested for astaxanthin, the red-orange pigment that makes salmon pink and lobsters red.

Astaxanthin sells as a nutraceutical and an aquaculture foodstuff, and as an ingredient in various cosmetics. These are valuable products. "Astaxanthin sells for something like $3,000 per kilogram," says Professor Chisti.

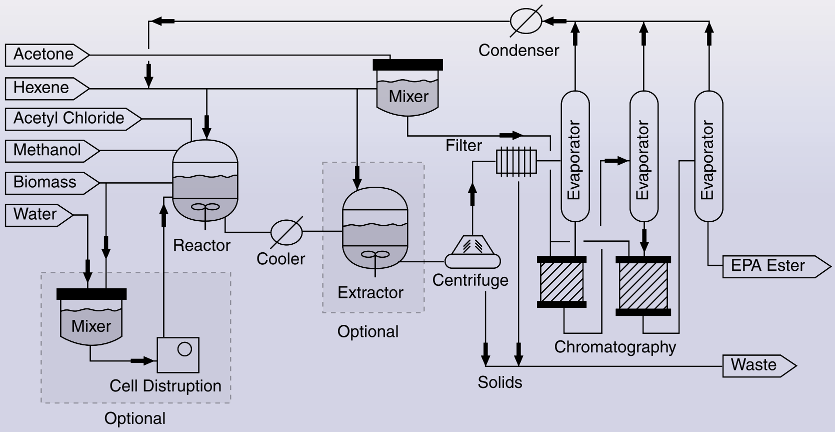
In aerial photos, the outdoor ponds used for growing microalgae look like vivid daubs of paint: iridescent green and dayglow orange.

In Almería, Professor Chisti began growing microalgae not in open ponds, but in a contained environment – a photobioreactor – away from all possible contamination.

At one end of the photobioreactor is an airlift pump to produce flows, while most of the reactor takes the form of looped series



At Almería in Spain a pilot photobioreactor cultures a microalga which will be harvested and processed to produce EPA, a valuable omega-3 oil. Although the extraction process – shown below – is sophisticated and costly, for high-value products the use of photobioreactors is approaching commercial viability.



of perspex tubes. Cultivating organisms that are light-dependent introduces another set of factors. Within the bioreactor there will be many different zones of illumination; where an organism sits within the culture will determine how much light it gets.

Strangely enough, one of the health discoveries of recent years – that eating fish seems to confer some protection against heart attacks – may in fact be attributed to microalgae. The omega-3 oils found in fish originate not in the fish themselves, but in the algae they feed on.

The fatty acids in omega-3 oils reduce blood clotting by decreasing the stickiness of blood platelets and, it is thought, may play a role in stabilising heart rhythms and reducing inflammation.

Professor Chisti's interest has been in the feasibility of producing one particular omega-3 oil called eicosapentaenoic acid (EPA) directly from microalgae. EPA and its derivatives have proved useful in preventing and treating coronary heart disease, abnormal cholesterol levels and several carcinomas.

Currently the only commercial source of EPA is fish oil, which fluctuates in price and quality, and can be subject to contamination with pesticides and heavy metals. Moreover, the demand for EPA is growing while the supply is fixed.

In Almería the photobioreactors were set up to culture the EPA-rich microalgae *Phaeodactylum tricornutum*. Typically for products like EPA 70 to 80 percent of the total cost of production will be in the processing and recovery. In this case processing and recovery meant three steps: extracting the fatty esters from the algae, separating EPA by pushing the dissolved fatty esters through columns of silver silica gel, and finally removing the chlorophylls. Professor Chisti and his collaborators calculate that producing EPA from microalgae will be cost competitive with fish-oil-based EPA if the algae can be harvested for less than US$4 per kilogram.

In 2000 Professor Chisti applied for his current job based at Massey's Palmerston North campus. He had enjoyed living in Almería, but never really came to grips with colloquial Spanish.

Today much of his research work is carried out in collaboration with former colleagues in Waterloo and Almería. He is soon to fly to Almería to conduct a PhD examination of a student whose work has been in producing the cholesterol-lowering drug lovastatin from gene recombinant moulds.

Professor Chisti's New Zealand-based postgraduate students are looking at such things as removing phosphate from wastewater and the enzyme treatment of hides to remove wool. Daniel Manderson, with whom this article began, is now in Ireland, where he will very likely end up employed by the biopharmaceutical industry, as happens to many of Professor Chisti's former students.

For Professor Chisti, biotechnology retains the allure and excitement that first attracted him as a student. In no other industry, he says, is there so close an alliance between knowledge and wealth creation, between commercial success and research expertise. Many biotech companies are pure research entities, contracting out the application of their research, and many universities end up spinning off biotechnology enterprises.

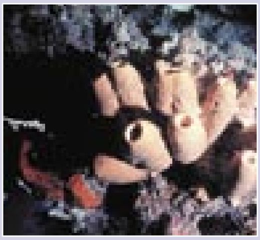
In the United States the biotechnology industry is again riding a wave of investor confidence. There have been company failures, as is to be expected, but the companies succeeding are doing so spectacularly well. "I have just been looking at a company called Invitrogen," says Professor Chisti. "In 1999 their revenue was around US$93 million; last year it was US$778 million!"

And all this is but a foretaste of what is to come. An editorial Professor Chisti has written for *Biotechnology Advances* is provocatively headlined 'Who needs a conventional dairy industry?'

"Why not microbial milk?" the editorial asks. Many of the soluble compounds in milk are already being made by recombinant organisms. Microbial milk would mean ground water uncontaminated by nitrates. It would be pesticide and antibiotic free. It would be cheaper too (though the branding campaign could pose problems).

Professor Chisti is being provocative – playing to the 'yuck' reaction – but his question is a proxy for those other larger ones: Where will biotechnology take us? How will it benefit us? What might it cost us?

Who is to say?



A few small problems

Find the protein that interests you, find the gene that produces it, take that gene and paste it into a helpful bacterium, and then produce any amount of the protein you like. It is a lovely concept beset by practical difficulties.

One of the difficulties is that organisms, such as *E. coli* and mammalian cells, produce different types of proteins. Many human and other mammalian proteins have carbohydrates attached to them, a condition termed glycosylation. Most of the proteins produced by bacteria are not glycosylated. So to produce many proteins you must use a mammalian cell line – and mammalian cells will divide only so many times before dying.

To get around this limitation, extensive use is made of hybridomas: cell lines created by fusing a cell that produces the protein wanted with a tumour cell, which will go on living and dividing long after an ordinary mammalian cell would have died.

However, even these techniques may not work where the target product is the result of complex biosynthetic pathways in cells that have a limited life.

Sponges have been of great interest to researchers looking for bioactive compounds. Sponges are soft, immobile and outwardly seem essentially defenceless, so you might expect that their chemical defences against predators would be highly sophisticated, and so it proves.

Two antiviral drugs already on the market, Acyclovir, a treatment for herpes, and AZT, employed against HIV AIDS, can track their lineages back to compounds isolated from a Caribbean sponge, *Cryptotheca crypta*, in the 1950s.

But generally researchers are having great difficulty in producing enough of the compounds of interest. Many arise not out of the action of one gene, but of many, so transferring a gene may not work. And, as with mammalian cells, growing sponge cells in a bioreactor is also difficult: after a certain number of multiplications the cells die.

The ideal, as with mammalian cells, would be to cross a sponge cell with a sponge tumour cell. But, according to Professor Chisti, who was part of a team that investigated the cultivation of sponges, no sponge tumour cells have yet been found and attempts to produce cancer in a sponge have been unsuccessful.

Industry links vital

Partnerships between universities and New Zealand's biotech sector are an ideal opportunity to ensure the critical needs of the industry are met, writes NZBio CEO Brian Ward.

Initiatives that encourage collaborations between universities and the biotech industry benefit both on a number of levels. They allow businesses and tertiary institutions to exchange knowledge, giving companies insight into research projects and researchers insight into the world of commercialisation.

Academic staff can provide expertise and skills to address the needs of companies as they develop. This is a great advantage for small firms, who, while they may be specialists in their own area, often don't have in-depth or detailed information about other topics.

Universities are also an important resource for continuing education, providing an opportunity for those already working in the industry to up-skill or broaden their field of knowledge.

As with any industry, the courses universities offer must be relevant to the biotech sector, and match its demands as these unfold. It is vital that training and development priorities and emerging trends are identified early enough to be incorporated into study programmes.

That is why it is essential that tertiary institutes and biotech companies continue to work together, to make sure we address any knowledge gaps and have the capacity to take part in and drive the industry forward.

By working together, students, university staff and established biotech firms strengthen their own networks, and diminish the sometimes perceived barrier between academia and 'the real world'.

These kinds of collaborations can provide opportunities for future employment, as well as giving students an idea of the career options available to them. Nothing beats practical experience for finding out how your skills fit the industry.

This is where a truly well-rounded education is so important. It is becoming increasingly apparent that to succeed in the biotech industry today, you need to have a variety of abilities, not just technical expertise, but commercialisation skills and business know-how.

While it will always be important to have world-class science capabilities, we should recognise that practical industry experience is also very valuable.

Brian Ward
CEO, NZBio