

## *Polluting natural gas turned into nutritional protein source*

by science reporter Mette Minor Andersen, Denmark

**A**re we capable to meet the expected demand for foodstuffs, particularly in the form of meat, milk and eggs over the coming years?

The challenges that increasing population provides are enormous.

In the next 25 years, the world will need to produce 250 percent more meat, milk and eggs than it does now. To help address this issues, UniBio Ltd has overcome demanding challenges and started production of a new protein source, Uniprotein® – a nutritional good, cheap protein source made from either natural gas or methanol.

Large-scale production of commodity chemicals by fermentation routes may well become one of the fastest moving enterprises of the early 21st century. Among the most promising processes is the production of animal feed from natural

gas; a cheap and abundantly available natural resource. UniProtein® is an example of a nutritional good, cheap protein source not genmanipulated and with a content of protein that is on the level of protein concentrates. Imagine one of the most polluting gasses turned into feeding stuff! That's what this product offers.

In January 2008, UniBio Ltd entered into a contract with the Republic of Trinidad and Tobago (T&T) for the production of UniProtein®. A pilot plant is built in Point Lisa's, Trinidad. Apart from local contributions, such as buildings, power, etc, UniBio Ltd together with IPU, Technical University of Denmark, GEA Scandinavian Liquid Contractors and Ramboll Ltd delivers the pilot plant as responsible subcontractors. The pilot fermentor has been running from March 2009 and in the summer of 2010 the production plant will be established with a capacity of 100,000 tons per year.

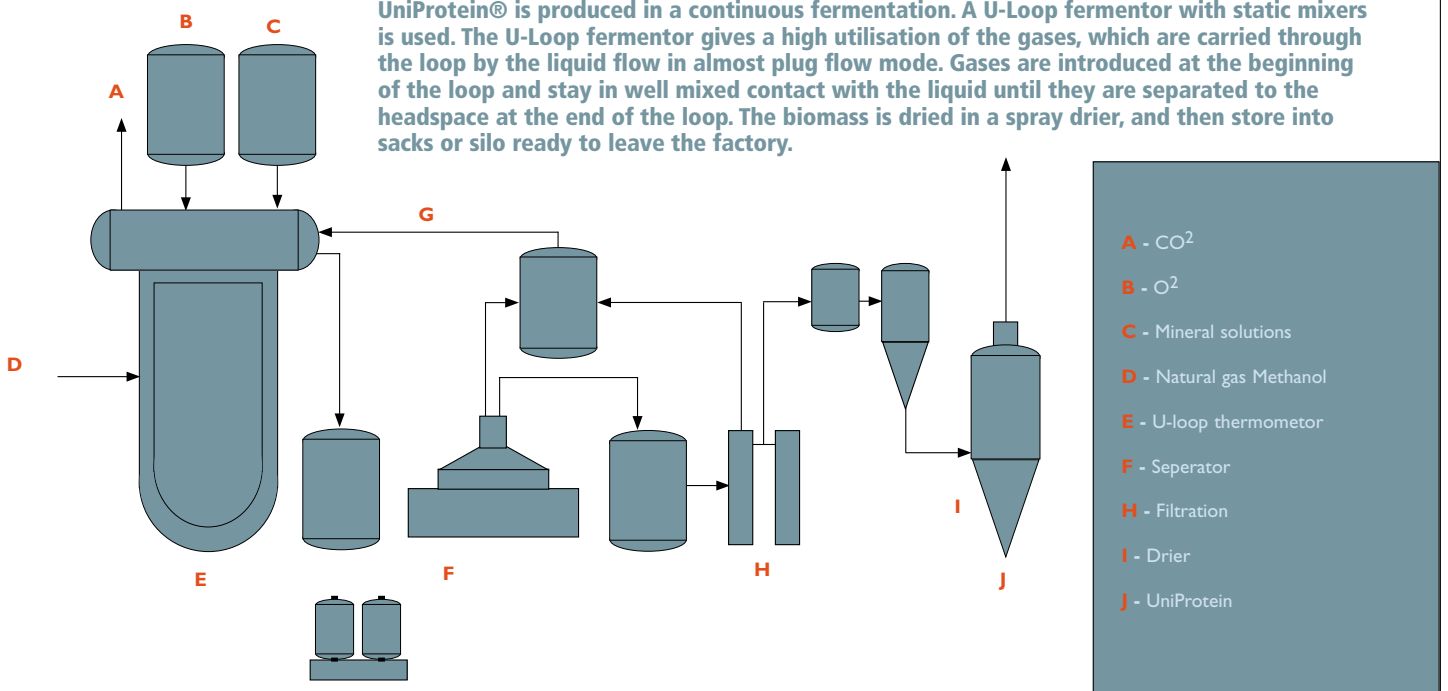
### Market potential and nutritional equivalency

If the product were on the market today, an estimated minimum demand would be in the order of six million tonnes per year. The annual production of industrially-produced feed is more than 630 million tonnes, equal to more than 125 million tons of 100 percent pure protein, equivalent to 175 million tons UniProtein® per year.

Currently animal feed rich in protein is produced from fishmeal, but a progressive over fishing of the oceans has lead to rapidly increasing prices of fishmeal, and as a result the price of fish from aquaculture is consequently being driven up with drastic consequences of grave nutritional problems in third world populations.

Methane from natural gas is used as a carbon source for a bacterial culture found in the natural habitat; in this case lakes. The only other raw materials are O<sub>2</sub>, NH<sub>3</sub> (or

UniProtein® is produced in a continuous fermentation. A U-Loop fermentor with static mixers is used. The U-Loop fermentor gives a high utilisation of the gases, which are carried through the loop by the liquid flow in almost plug flow mode. Gases are introduced at the beginning of the loop and stay in well mixed contact with the liquid until they are separated to the headspace at the end of the loop. The biomass is dried in a spray drier, and then store into sacks or silo ready to leave the factory.



ammonia salts) and a few other minerals. The resulting product is at least nutritionally equivalent to high-quality fishmeal (LT) and furthermore is free from dioxin, Aflatoxin, Ochratoxin and heavy metals.

The final product is a bright, brownish granulate, of uniform quality and with a P-content much lower than fishmeal, thus giving rise to no environmental problems and better productivity in fish production.

The environmental influence of UniProtein® is positive as each kg will replace five kgs of freshly caught wild and transformed into fishmeal, to produce a kg of growth of fish from aquaculture. As almost 50 percent of consumer fish is produced from aquaculture, this has caused enormous over fishing for industrial use.

## Protein Manufacturing

UniProtein® production process is aerobic with natural gas as the carbon and energy source. Industrial pure oxygen is used for an oxygenation fermentation process and ammonia is used as the nitrogen source.

In addition to these substrates, the UniProtein® culture requires water, phosphate and several minerals including magnesium, calcium, potassium, iron, copper, zinc, manganese, nickel, cobalt and molybdenum. Sodium hydroxide and sulphuric acid are used for pH regulation. All chemicals are of food grade quality. Phosphate is supplied as phosphoric acid, the minerals as sulphates, chlorides or nitrates. The pH is regulated to 6.5+/-0.3 and the temperature is kept at 45°C.

A high biomass concentration with a high metabolic activity throughout the fermentor ensures that methane and oxygen are rapidly utilised as soon as they are dissolved in the fermentation liquid. The overall gas utilisation in the loop fermentor can exceed 90 percent.

After each campaign of a minimum of four-to-five weeks continuous operation, the fermentation system is cleaned with hot sodium hydroxide followed by a short treatment with a dilute nitric acid solution and sterilisation with steam at 120°C for one hour. The fermentor is filled with water, which has been heat sterilised at 130°C for ten seconds. Gases and solutions of minerals, ammonia and phosphoric acid are all sterile when fed into the fermentor.

Addition of the different nutrients is regulated according to consumption. The



## licences

The purpose of UniBio Ltd is to sell licences to fermentation and produce UniProtein®. It is also the aim of the company to develop techniques and methods of fermentation, in the most efficient way, that assists the sale of licenses and know-how globally and establish UniBio-industries with its own production, educational and developmental activities.

A factory producing 100,000 tonnes per year is estimated at about US\$125 million. Furthermore, it is the aim of the company to continue the development of the production process, to target the product and to develop new applications of UniProtein® and to extract from the product amino acids, enzymes, aroma and flavoured additives.

Production units can be established in all areas including environmentally sensitive ones such as Alaska, as there is no emission of polluting fluids or gasses, but only clean, cool water and vapours.

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# Protein

## Bacteria M102 from a lake in Germany

For several years UniBio Ltd and IPU, an independent company located at the Technical University of Denmark, has cooperated closely on the development of a fermentation plant to the production of protein-rich and nutritious product called UniProtein® (a single cell protein). Further research, headed by Unibio Ltd, with financial support from the Danish Ministry of the Environment and Energy, the Danish Engineering company Ramboll, IPU and the department DTU Biosys at Technical University of Denmark have led to qualitative improvements of the reactor design and fermentation process. The company has now started production and is focused further on a targeted development of UniProtein®. However, it all started at a lake in Germany, Plöner See, in the beginning of the 1980s where German researchers from the Max Planck Institute isolated the now patented methanotropic (metamorphic) bacteria M102. They found the bacteria in six-to-eight meters of water, where it feeds on methane, which rises from the bottom of the lake as marsh gas.

At that time Ebbe Bush Larsen, now UniBio's CEO, was in contact with Dr M Nagub and Professor Jürgen Overbeck from the Max Planck Institute. They had done the basic research of the M102 bacteria.

In 1982 Ebbe Bush Larsen bought the patent of the bacteria and know-how from the Max Planck Institute, and this was the start signal of the foundation of the company Dansk BioProtein A/S in 1985. Soon after they entered into cooperation with Technical University of Denmark, DTU, and University of Southern Denmark in order to evolve the best growth conditions for the organism.

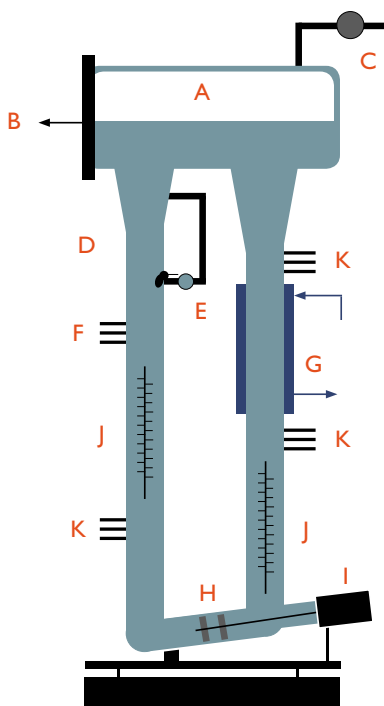
In the period 1986-1990 they developed the technology in cooperation with DTU and with economic support from The Technology Agency. At first it was a 500 litres plant in Odense, Denmark, which was enlarged several times, ending up with a newly developed 20m<sup>3</sup> U-loop fermentor.

Subsequently, patents were applied for, lately in 2001. The development continued in cooperation with DTU. The aiming of the optimisation of the plant, with considerable support from The Danish Energy Agency, resulted in the seven metres high U-loop fermentor. It was set up at the department DTU Biosys Pilot Plant, in the project period 2000-2004. From 2004-2005 the Danish Energy Agency supported a project on process optimisation, too. The total energy saving with the new technologies and optimisation is about 50 percent compared to the traditional fermentors.

control system includes on-line measurements of headspace gases and the ammonium concentration in the medium. Feeding of mineral nutrient solutions is programmed according to a detailed knowledge of the stoichiometric requirements and UniProtein® culture for the different minerals.

The continuous fermentation is operated with two-to-three percent biomass (dry matter) and a dilution rate of 0.20 to 0.25h<sup>-1</sup>. The biomass of the harvest is concentrated to over 15 percent by centrifugation and then to more than 30 percent by ultra-filtration (UF).

The concentrated biomass is quickly heated to 140°C in UHT (Ultra High Temperature) unit in order to obtain a sterile product followed by a quick cooling to approximately 70°C. In the process the biomass is inactivated and the cell undergoes lysis so that the protein becomes more accessible.

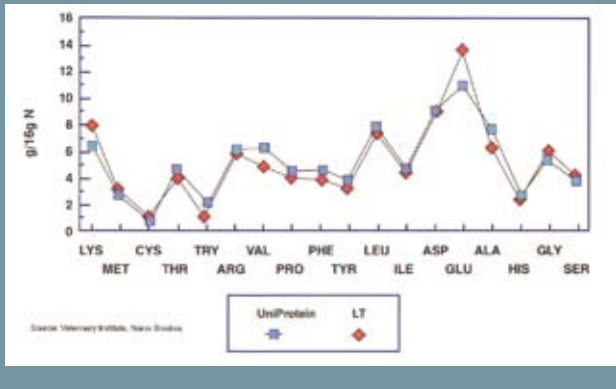


### Components of the U-Loop fermentor

- A - Degassing unit
- B - Continual harvest of biomass
- C - CO<sub>2</sub> outlet
- D - Two-phase nozzle, where fluid from the fermentor is mixed with air and then reinjected into the fermentor, to achieve small bubbles
- E - Nozzle pump
- F - Injections of medium
- G - Cooling
- H - Propeller pump for moving the fluid through the loop
- I - Engine for pump
- J - Static mixers. Prevent bubbles from merging while moving through the loop
- K - Additional injection points

The design of the fermentor is one of the more important aspects of the process. In traditional stirred fermentors there are problems with achieving sufficiently small bubbles (large surface area of the gas) and there is a large energy waste due to inefficient stirring of the bulk liquid. Furthermore, there are problems with cooling when upscaling. The fermentor, that is to be used in the Unibio factory, is based on the patented U-Loop fermentor, developed in cooperation with the Danish Technical University (DTU). "In the U-Loop design we have achieved a much larger surface area and mechanical stirring is no longer needed. A 50m<sup>3</sup> nozzle-loop fermentor and a 20m<sup>3</sup> U-loop fermentor have been built and tested by scientists from DTU," says Ebbe Bush Larsen. Apart from being much more effective – 4.0kg/m<sup>3</sup>/h against <1.8 kg/m<sup>3</sup>/h – than traditional fermentors, there is about 30 percent energy saving in the U-Loop fermentor.

### Amino acid profile in UniProtein® and Lt-fishmeal



Finally, the UHT-treated biomass is dried in a spray dryer with an integrated fluid bed. This gives a non-dusty agglomerated product.

In order to minimise the use of process water and to minimise the amount of wastewater, process water from the centrifuges and ultra-filtration is returned to the fermentor after a short heat treatment. The product is routinely examined for microbial contamination, water content and chemical composition. Tests have shown that the UHT treatment kills all the bacteria used in the production of UniProtein®.

Spray drying is the last step in the production of UniProtein®. It undergoes no other technical processes of preparation before use.

### The market for protein food

The global production of animal feed is approximately 630 million tonnes per year. Parallel to the annual increase in the global population of approximately 1.3 percent the need for animals for human food and subsequently the need for animal feed increases. The annual increase in animal feed production has thus averaged 1.8 percent over the last 10 years. The amount of pure protein in animal feed is on average 20 percent which amounts to a global demand of more than 125 million tonnes of pure protein a year. The market for UniProtein® is approximately 175 million tonnes.

The low moisture content, the fact that it is not hygroscopic and the low level of unsaturated fatty acids makes UniProtein® a chemically stable product which can be stored for a long period of time. UniProtein® is easily mixed into other compounds. A method for reduction of nucleic acids to less than one percent has been developed.

The amino acid profile of the protein content is nutritionally favourable with a high content of the important amino acids cysteine, methionine, threonine, lysine, tryptophane and arginine of 0.7 percent,

3.1 percent, 5.2 percent, 7.2 percent, 2.5 percent and 6.9 percent, respectively, expressed as percent amino acid of total amount of amino acids.

The fatty acids are mainly the saturated palmitic acid (approximately 50 percent) and the monounsaturated palmitoleic acid (approximately 36%). The mineral content of the product is favourable with high amounts of phosphorus (approximately 1.0 percent w/w), potassium (approximately 0.4 percent w/w) and magnesium (approximately 0.2 percent w/w). The product is virtually free from heavy metals due to the controlled production process and the fact that all minerals used are food grade.

### The market for human use

Proteins for human consumption are a much differentiated market with a large range of different types of protein products in respect to raw materials, process and concentration. For several types of protein



Diagram showing one floor of the plant

the concentration ranges from 'raw' non-concentrated to 'isolates' with 90 percent-plus protein content.

Protein addition in the food industry is primarily done to achieve a functional effect (mouth feel, water binding, fat stabilisation, adhesiveness, etc) and a nutritional effect (protein supplement, increasing biological value of other proteins, increase fullness, etc). The

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# Protein

functional effects are in demand by the meat and dairy industries, while the nutritional effects are used in baby food, diet drugs, athlete nutrition, etc.

The Royal Veterinary and Agricultural University of Denmark conducted tests with a panel of 24 people who were given a breakfast cereal containing 10-50g and 75g respectively. The panel was tested for allergy reactions, etc. No adversary effect of the bioprotein was reported.

## Teaching Biotech at the University of Trinidad & Tobago

UniBio Ltd and researchers from the Technical University of Denmark are helping to expand the University and Trinidad and Tobago's educations in biotechnology. T&T is actively pursuing new high tech solutions to ensure continuing and future economic and social development of the country. Skilled and highly educated manpower is needed for these enterprises, which can be supplied locally by exploiting international collaborations such as with the Danish company UniBio A/S and researchers from the Technical University of Denmark.

An impressive example of the country's commitment is the establishment of the University of Trinidad and Tobago, UTT, over the last four years, which now has a broad palette of education, at a number of campuses around the country. Large investments by government are being made in infrastructure including state of the art campuses and teaching methods as well as the equipment needed to make UTT the leading university in the Caribbean.

One of these investments in education, which has direct application to new future industries on Trinidad, is the establishment of a substantial biotech focus in the Bachelor of

## The product

The content of protein in the biomass is on the level of protein concentrates (approximately 71-plus percent in dry form) and no further concentration of the protein content is planned. Apart from protein the biomass contains fat (~10 percent), ash (~7 percent), carbohydrate (~7 percent), fibres (<1 percent) and nucleic acids.

UniProtein® is well suited as a direct feed for animals with a short life span. If it is used as additive for humans or for animals with long life spans, the products content of nucleic acids in the UniProtein® will become a problem, since it can cause kidney stones and bladder stones. To offset this the nucleic acids are neutralised through hydrolysis without loss of protein. After this process the product can be used as additive for human food. The taste is neutral, and even a - nutritionally very poor - diet of corn porridge can be turned into an excellent diet.

Other products that can be derived from the hydrolysed primary product are flavour enhancers - can replace the now forbidden 'Bovril' derived from bonemeal. A long range of adhesives can also be produced from the hydrolysed product giving Unibio A/S access to a profitable market outside the food sector.

UniProtein® has been tested as feed for salmon, calves, pigs and chickens with positive results for both growth and accept. Furthermore an increased resistance to disease was seen, when feeding salmon with UniProtein®.

**1: An analysis of basic UniProtein® on dry weight basis gives the following mean values**

Composition	Percent
Crude Protein	70.6
Crude fat	9.8
Ash	7.1
Crude Fibre	0.7
N-free extract	11.8
<b>TOTAL</b>	<b>100</b>

## Amino Acid Composition

Cysteine	4.5g/kg
Methionine	19.8g/kg
Threonine	32.8g/kg
Lysine	4.6g/Kg
Tryptophane	15.7g/Kg
Leucine	54.9g/Kg
Isoleucine	33.6g/Kg
Valine	44.6g/Kg
Histidine	18.0g/Kg
Arginine	48.3g/Kg
Alanine	51.9g/Kg
Aspartic acid	65.1g/Kg
Glutamic acid	77.1g/Kg
Glycine	35.7g/Kg
Proline	31.7g/Kg
Serine	26.6g/Kg
Tyrosine	26.0g/Kg

Nutritional properties	Technical properties	Other properties
Neutral taste and no smell (flavour)	Consistency in analysis and quality, chemically and physically	Immune stimulating effect
Good palatability	Uniform particle size	Infested fish show reduced mortality using UniProtein®
Increased growth and good feed conversion	Independent of seasonal changes	
	Total process control	
	Good handling characteristics	
	Long shelf life/chemically stable	
	Good binding properties	

Science and Masters programmes in Process and Utilities.

Life sciences and bioprocess engineering subjects are now taught and with the construction of a cutting edge biotech pilot plant and laboratories at the Point Lisa's campus, the students will soon be able to apply their theoretical knowledge in practical classes at the undergraduate and postgraduate level.

The pilot plant and laboratories are currently being built in collaboration with UniBio A/S and the Technical University of Denmark. It will also provide a demonstration and testing ground for local and international industry wishing to examine the potential of biotech processes employing natural gas as

the primary carbon source. Building of the pilot plant provides an enormous scope for student projects and education in biotech as well as other engineering fields such as process control.

Right now the pilot plant at point Lisas, Trinidad, is running and soon a full production plant will produce 100,000 tonnes of UniProtein per year. Together with E-Teck, NEC, UTT and the government of Trinidad and Tobago, UniBio Ltd has entered the market in order to make it an important player on the world protein market.

### MORE INFORMATION

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