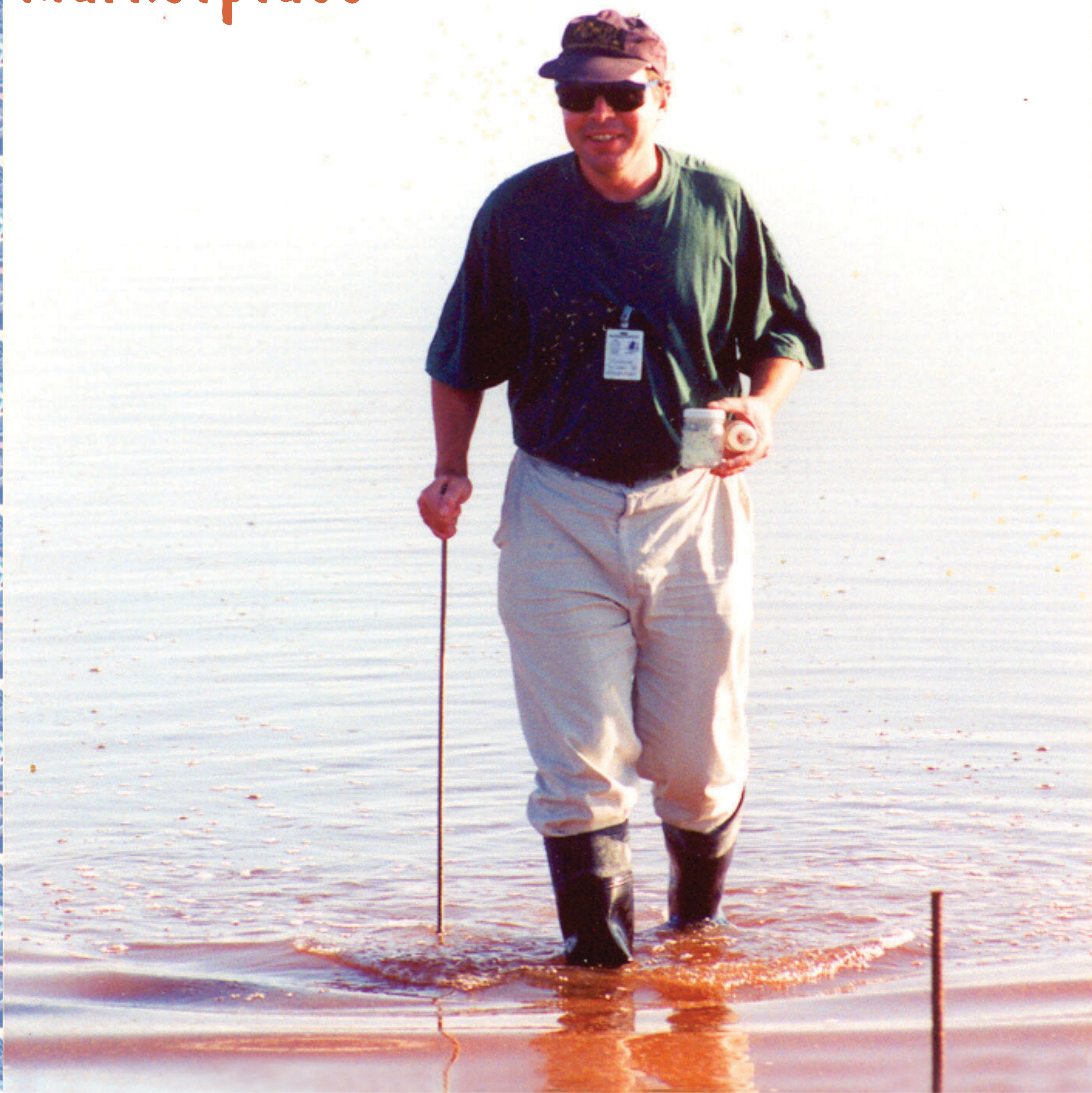
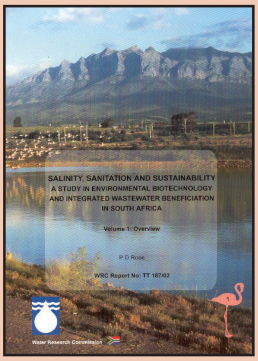
From the laboratory to the marketplace

Dunaliella is a green, salt-loving micro-alga that revels in hyper-saline water, harsh sunlight and extreme heat. It is also the Ingredient-X in cost-effective biological mass-production of high-value β-carotene - an anti-oxidant that is converted to Vitamin A by the liver. Environmental biotechnologist Peter Rose and his team came up with a process that uses polluted wastewater as a growth medium for this important harvest, reports Catherine Knox. Their initial laboratory work has been scaled up for evaluation as a commercial production process.



Part three of a series of good news water stories taken from Peter Rose's 12-volume report on an integrated WRC-funded project. Professor Rose is the director of Rhodes University's Environmental Biotechnology Unit (EBRU).



In Africa now, a sustainable process is one that pays for itself – this is one of the central tenets of a 10-year Water Research Commission (WRC)-funded research project initiated and led by Peter Rose into the bio-remediation of polluted water.

Rising salinisation caused by industry, agriculture and natural phenomena, poses a profound threat to South Africa's water resources. For Professor Rose, one of the first steps in helping to do something about this situation was re-imagining the threat as an opportunity. He is the head of Rhodes University's Environmental Biotechnology Unit (EBRU) and thanks to his vision, the integrated WRC project has developed biological methods that produce hard commercial benefits at the same time as they effectively de-salinate wastewater.

During the initial project which involved harvesting *Spirulina* as a side-benefit of the biological clean-up job in the meso-saline waste-stabilisation ponds at a tannery near Wellington (reported in *Water Wheel* Jan-Feb 2003), Rose's team of researchers noticed intermittent blooms of β-carotene-producing *Dunaliella* in the hyper-saline compartments of the ponding system.

The growth of *Dunaliella salina* was evaluated in a range of different effluent streams discharged by the tannery and the results were reported by MSc candidate Richard Laubscher in 1992. It transpired that hide soak liquors, generated in the process of rehydrating salt-cured hides, were *Dunaliella*'s preferred growth media. Cultivated in this heavy soup, the algae out-performed cell growth yields in custom-designed inorganic growth media developed specifically for *Dunaliella*. This was a surprising result for a photosynthetic organism apparently only able to grow in sunlight. The algal growth was accompanied by substantial reductions of organic load in the effluent.

In order to protect itself from fluid loss by osmosis in hyper-saline medium, *Dunaliella* produces glycerol – a phenomenon Rose and his team felt should be investigated as a possible contribution to the performance of the organic hyper-saline high-rate algal pond. The stimulation of microbial activity in the algal-bacterial co-culture could contribute to the efficient break-down of complex organic compounds observed in the treated effluent.

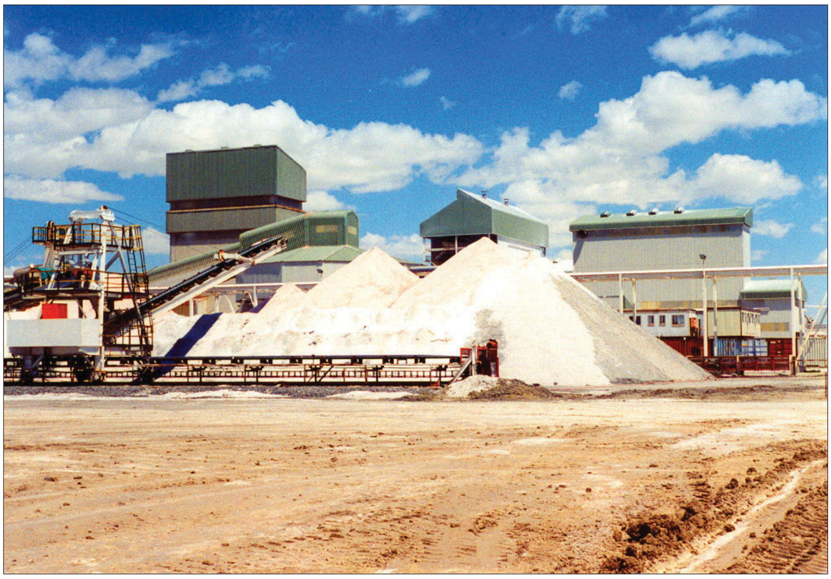
Robin Emmett, another of Rose's MSc students, studied glycerol production by a range of 15 *Dunaliella* species. She showed that the optimal glycerol production in tannery effluent was as much as four times higher than the sustained production reported for inorganic media. A series of flask studies showed that the amino acid glycine, present in high concentrations in hide tissues, was associated with the induction of glycerol release by *Dunaliella* growing in tannery effluent.

But while *Dunaliella* cultures yielded bright green luxuriant growth when cultivated in the

The Dunaliella *high-rate algal pond pilot plant treating saline carbonate brines at Botswana Ash Co, Sua Pan.*

EBRU researcher Richard Laubscher wades out to the collection point in one of the huge shallow evaporation ponds in the soda ash plant at Sua Pan in Botswana.

In a sun-catching bivouac high on a rooftop at UCT, chemical engineering PhD candidate Njodzi Zizhou is researching the use of the Dunaliella *high-rate algal pond methodology with other carotenoids.*



Organic contamination due to algal action threatens the economic viability of soda ash production at Sua Pan.

tannery medium, the downside was that β-carotene production was found to be extremely low under these circumstances. Nevertheless, when cells were transferred to a nitrogen-deficient stress medium, β-carotene production kicked into overdrive with an accumulation of the valuable chemical as high as 14% cell mass.

At this point the research team got another surprise: they found that the higher the organic strength of the original medium, the higher the production of β-carotene when cells fell on hard times in the stress medium.

Experimental studies in the laboratory and at Sua Pan in Botswana made it seem highly likely that a *Dunaliella*-based high-rate algal pond process for treating hyper-saline wastewater was worth scaling up in the field for further evaluation, but this has yet to be done as the EBRU team were focused on their second observation to do with β-carotene production.

They designed a dual-stage process that was patented by the WRC in 1992. This process involves a growth stage where optimal biomass flourished in hyper-saline organic wastewater. Cells from this initial "crop" are separated and transferred to a second stage where the medium is nitrogen-deficient, resulting in cell stress and the consequent production of optimal quantities of β-carotene. The dual stage process offers a "have-your-cake-*and*-eat-it" scenario with

One of the outcomes of the EBRU's Dunaliella *work is a collaboration with Professor Sue Harrison of UCT's Department of Chemical Engineering – her group is investigating other uses for the technology.*

maximum cell growth and maximum β-carotene production. Detailed follow-up studies were carried out by three of Rose's PhD students – Trevor Phillips, Lesley Horne Phillips and Malcolm Logie.

Trevor Phillips' work showed that high light intensity was an essential co-requisite for optimal production.

After graduating, Phillips went on to work at Sasol Co who funded the scaling up of the project in facilities at Sasolburg. Studies at this stage included the evaluation of cell-harvesting techniques. The dual stage process was then subjected to extensive and detailed evaluation in pilot-scale studies in a custom-built plant in Upington.

The CSIR has taken over ongoing research into the commercialisation of this method of producing β-carotene. Raj Laloo, another of Peter Rose's ex-student heads the project under the CSIR's Environmental Biotechnology programme manager Dusty Gardiner.

SUGAR IN THE SALT - UNIQUE COMBINATIONS FOUND IN *DUNALIELLA* CULTURES

Evaporation ponds used for the production of salt, soda ash and other evaporates are particularly susceptible to blooms of salt-loving (halophilic) algae and bacteria and this often results in high levels of organic contamination of the final product.

In 1991 Soda Ash Botswana (Pty) Ltd set up a solar evaporite production facility at Sua Pan in the Makgadikgadi region of central Botswana. Shortly afterwards, an accumulation of high levels of organic materials started to contaminate their final product. And so it happened that Prof Peter Rose's environmental biotechnology group got the chance to evaluate the performance of the *Dunaliella* high-rate algal pond in the treatment of solar evaporate brines.

Preliminary evaporation pond ecology studies showed that *Dunaliella* was involved the process that was probably driven by the high levels of sulphide, phosphates and nitrates in the brine pumped into the evaporation ponds from wells in the pan. The combination of hyper-saline carbonate brines, blinding sunlight and searing temperatures acted as stress-factors on *Dunaliella* that reacted by producing large quantities of extracellular polymeric substances.

Patricia Masemola, one of Rose's MSc students, reported in 2000 on nuclear magnetic resonance studies which confirmed that *Dunaliella* was implicated in the contamination of soda ash at Sua Pan. Unique sugar combinations were found in the exopolysaccharide produced by *Dunaliella* cultures in laboratory flasks, pond brines and in the final soda ash produced.

The study included the evaluation of a *Dunaliella* high-rate algal pond that could be used to strip both nutrients and contaminating organics from the evaporation ponding process. Reducing nitrogen and phosphate levels was shown to be an effective way of manipulating microbial growth in the system. The growth of *Dunaliella* was substantially inhibited in the brines that had been treated in the high-rate pond.
"In other words," says Prof Rose, "where *Dunaliella* grew in an uncontrolled manner the organics problem resulted. We found that if we used the *Dunaliella* ponding process to remove the growth nutrients ahead of this stage in the system, then downstream growth was prevented. The β-carotene recovery was a high-value spin off of this treatment operation."

The pilot plant study has not yet been followed up with the full-scale application, but it was shown that the process could play an important role in the management of algal production in saline wastewater impoundments, specially where solar evaporates are to be produced, either as a product or for the linear removal of contaminating salts from the water system.