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## Production of a free-enzyme catalyst for bioremediation using high cell-density fermentation

**Dr. Andrew Rodgers**  
**CSIRO Molecular and Health Technologies**  
**BPN Conference - 1<sup>st</sup> October 2009**

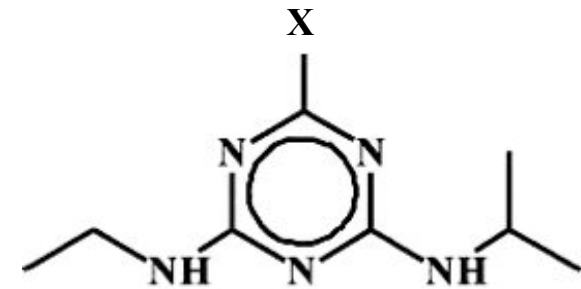
# Production of a free-enzyme catalyst for bioremediation using high cell-density fermentation

## Overview

- **Triazines are a very commonly used class of herbicide, despite some environmental concerns**
- **Bioremediation using free-enzyme catalyst is an attractive solution to the problem of waterway contamination**
- **A method for producing large quantities of a triazine degrading enzyme has been developed using high cell density fermentation**
- **Field trials have been highly successful**

# Triazine herbicides

- 4-(ethylamine)-6-(isopropylamine)-1,3,5-triazines are a class of herbicides widely used in agriculture throughout the world



- Triazines act by blocking photosynthetic electron transport
- Used against broadleaf and grassy weeds in major crops



Nebraska,  
USA

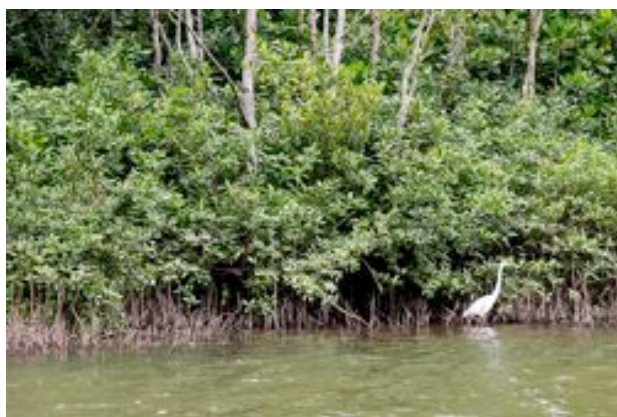
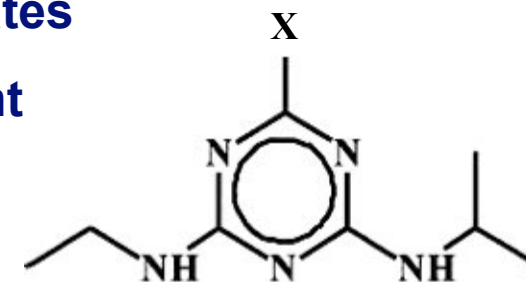


Queensland,  
Australia



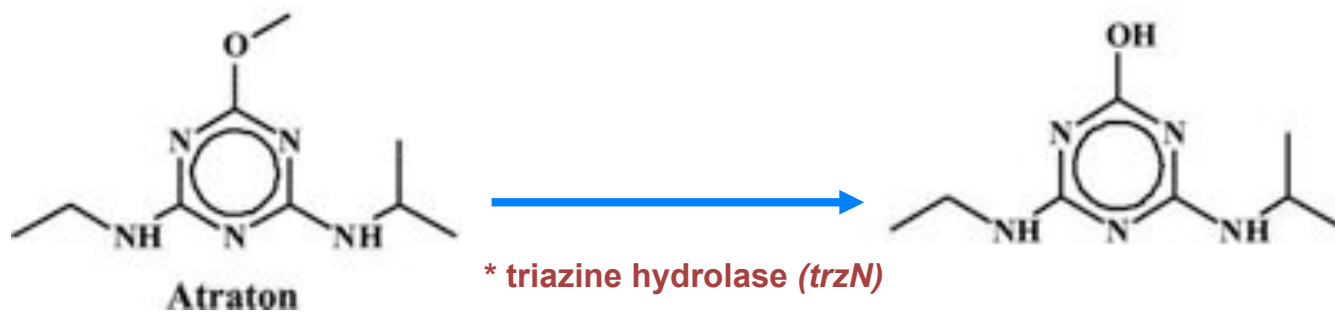
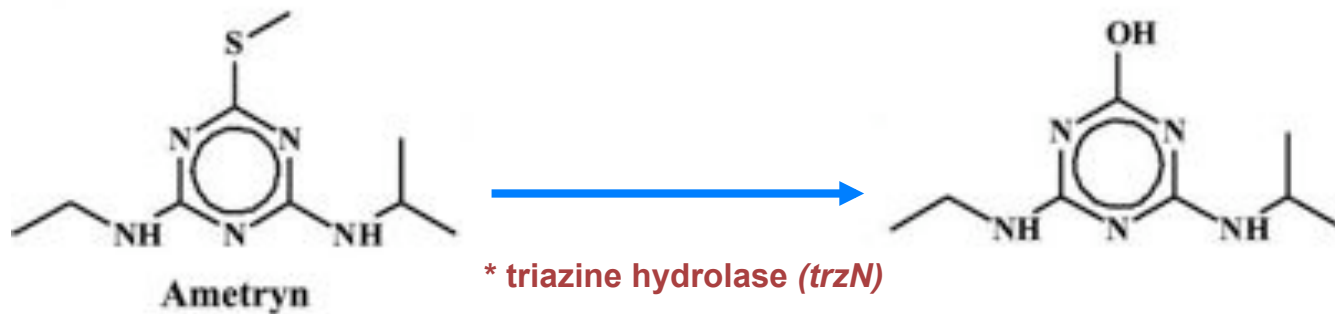
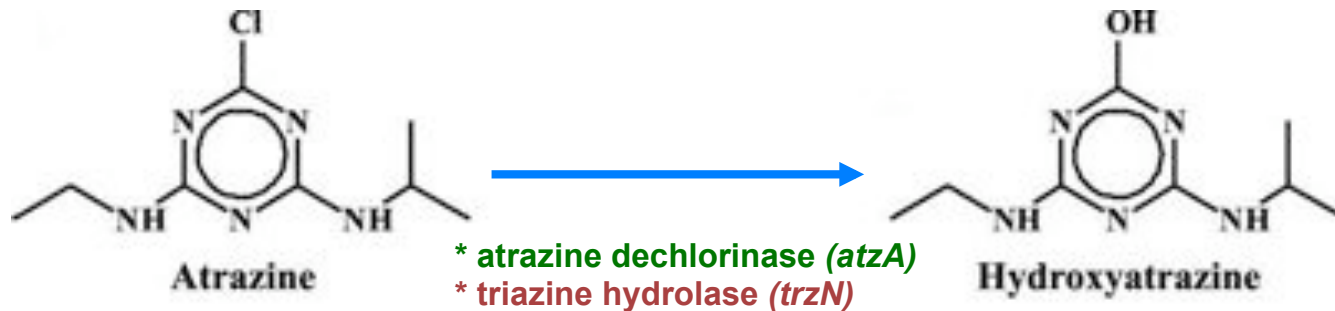
# Triazine herbicides: Environmental concerns

- causally linked to endocrine dysfunction in vertebrates (demasculation of frogs) at environmentally relevant concentrations (as low as 1 ppb)
- toxic to non-target photosynthetic species (bacteria, algae, mangroves and corals)



- persist in the environment ( $\frac{1}{2}$  life of 4-57 wks in soil)
- have been detected in surface and ground waters in several countries at concentrations up to 1 ppm

# Triazine herbicides: Biodegradation



# Strategy for bioremediation using triazine hydrolase

- Free enzyme is attractive as a bioremediant as issues surrounding the release of transgenic organisms are avoided
- Filtered lysate containing recombinant protein may be used in field trials free of any regulatory requirements



# Strategy for bioremediation using triazine hydrolase

Cloned the gene for triazine hydrolase from *Nocardioides* sp strain C190 (*trzN*) into an *E. coli* expression system (pET vector / BL21 host)

Optimize growth of recombinant *E. coli* in fermenters at 2-litre scale

Scale-up of fermentation at 100-litre scale

Cells homogenized and lysate filtered

Bioremediation field trials

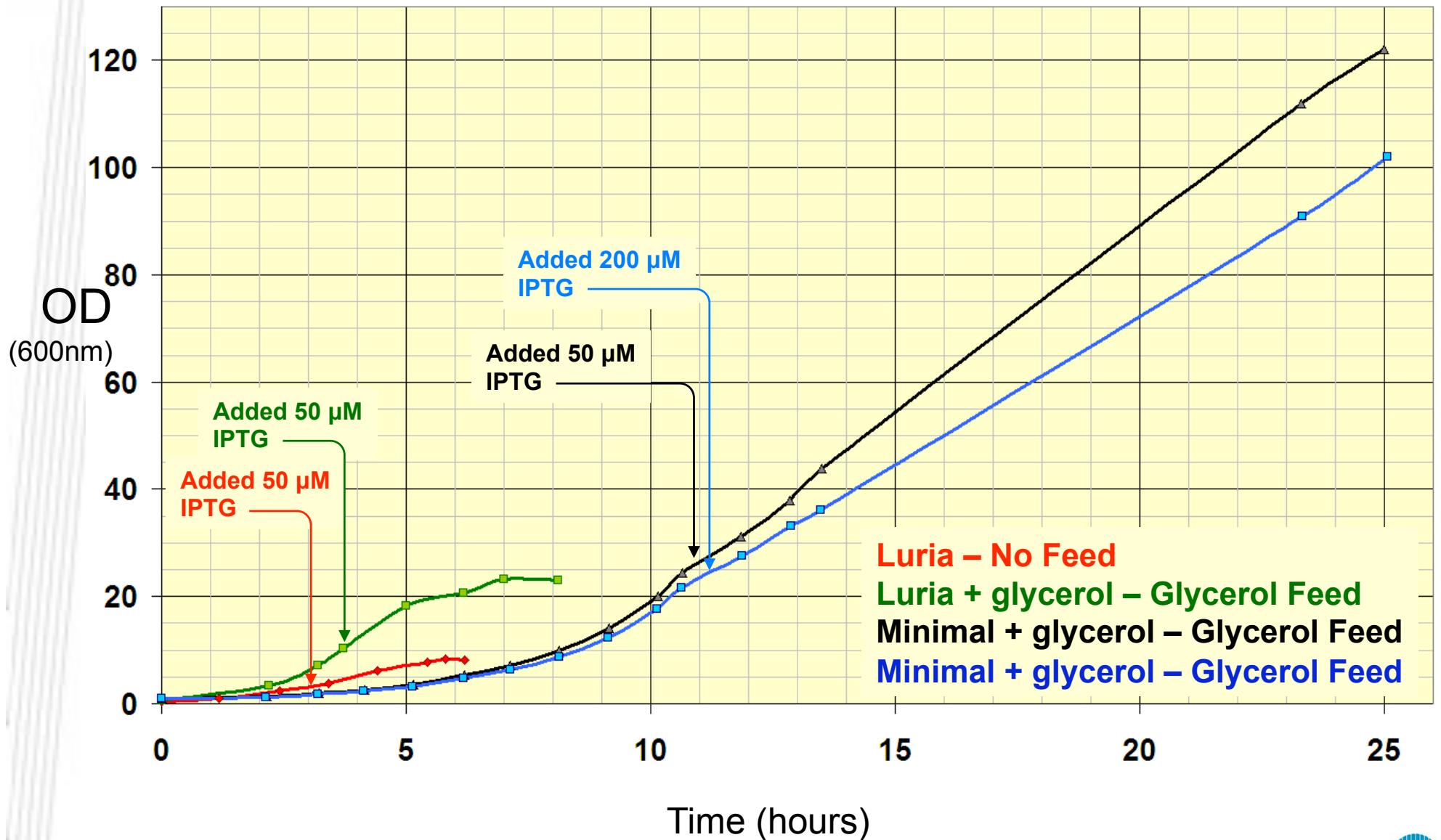
# Expression of triazine hydrolase (*trzN*) in *E. coli*

2-litre fermenters used to test growth performance of recombinant *E. coli* under 4 different conditions:

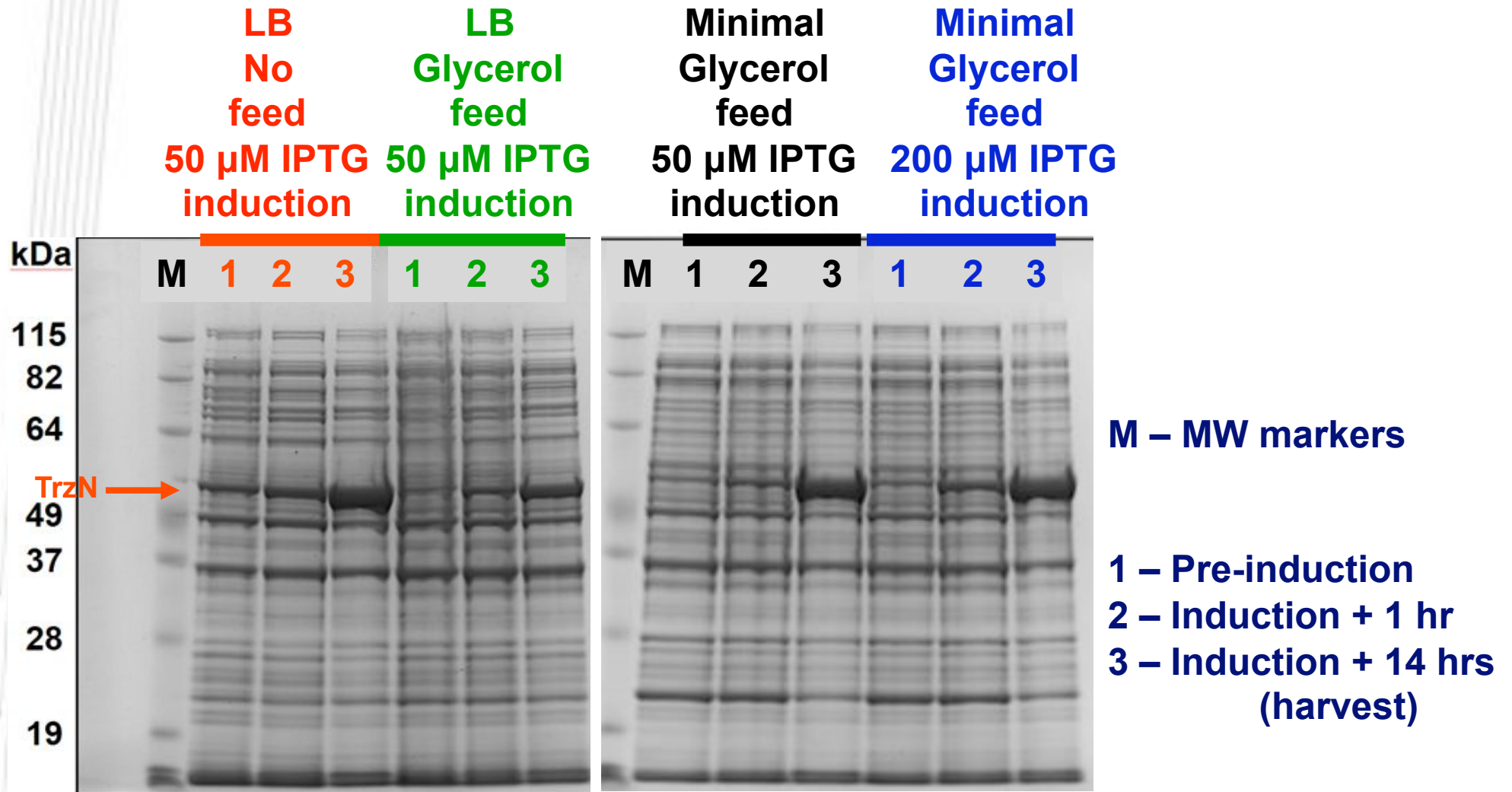
- Do cultures grown in rich (Luria) or minimal medium achieve higher cell densities?
- Effect of low (50  $\mu\text{M}$ ) or high (200  $\mu\text{M}$ ) IPTG to induce protein expression



# Expression of triazine hydrolase (*trzN*) in *E. coli*



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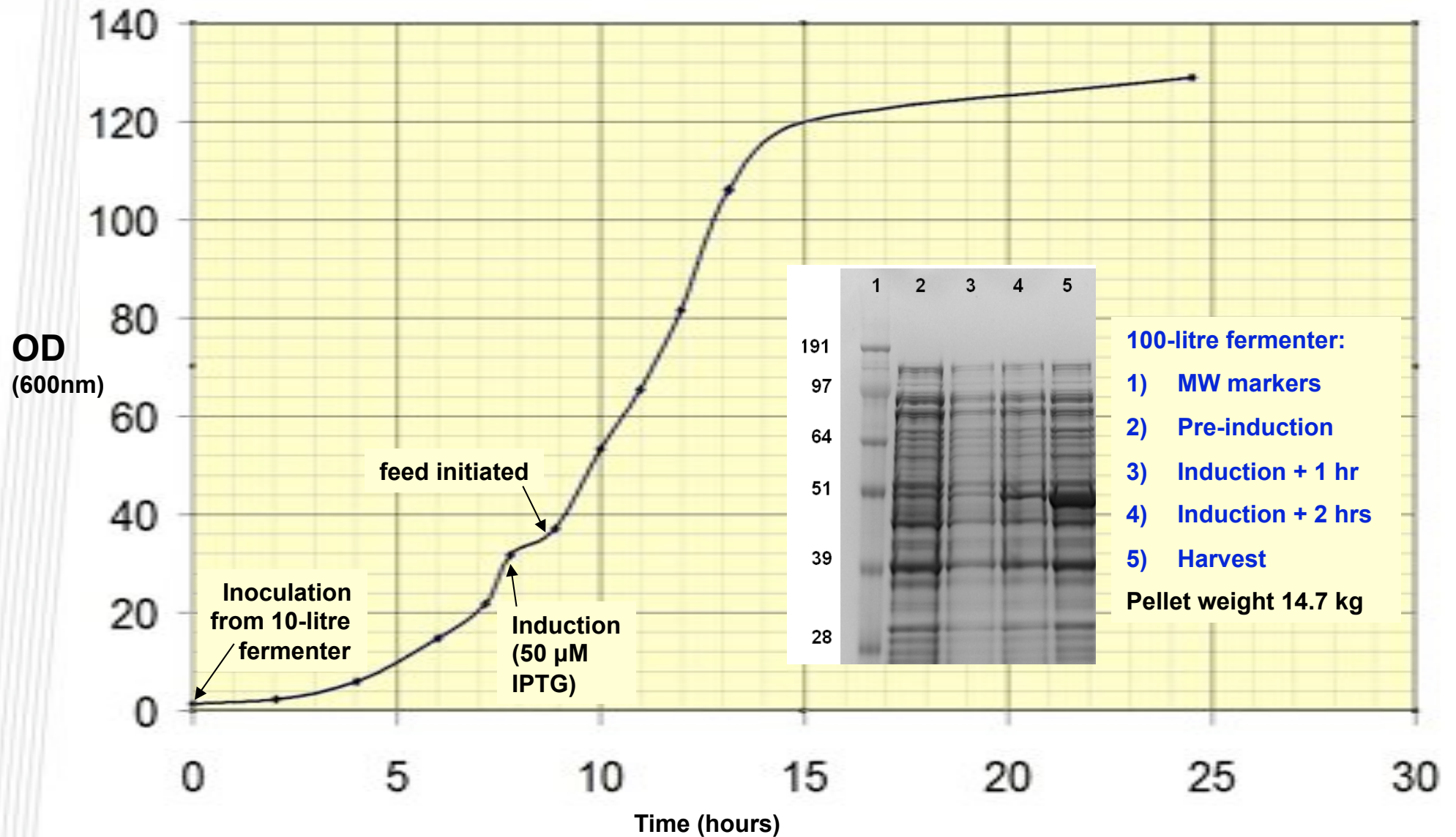
## Scale-up:

To produce a large quantity of cell lysate containing triazine hydrolase (sufficient for field trials) a 100-litre fermentation was carried out:

- Minimal medium
- Glycerol feed
- Induction with low (50  $\mu$ M) IPTG



# Expression of triazine hydrolase (*trzN*) in *E. coli*: Scale-up to 100 litres



# Preparation of filtered *E. coli* lysate containing triazine hydrolase (*trzN*)



- Cells were washed and resuspended in 100 mM MOPS, pH 6.9
- Cells were disrupted by 3x passage through homogenizer
- Lysate filtered through 0.22  $\mu\text{m}$  filter

# Bioremediation field trial with filtered *E. coli* lysate containing triazine hydrolase (*trzN*)

- A ~1.5 ML holding dam at a sugar cane farm near Clare, Queensland, was filled with headwater from irrigation of a field pre-treated with the recommended dose of atrazine (3.3 kg per hectare).
- After filling, atrazine concentration in the dam was 170 µg/L

An atrazine contaminated holding dam



# Bioremediation field trial with filtered *E. coli* lysate containing triazine hydrolase (*trzN*)

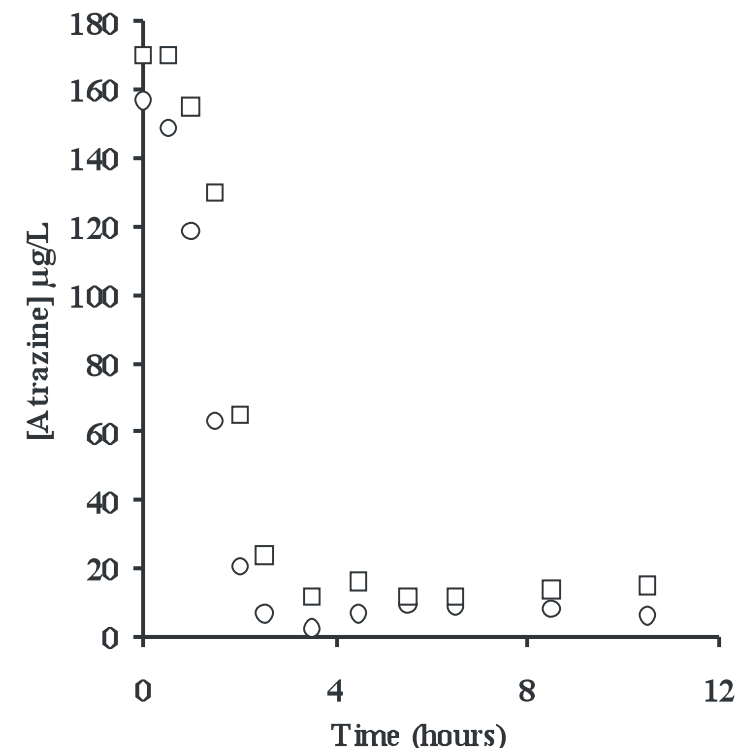
- 240 g of lysate in 20 litres of water, applied by hand, spreading evenly across the surface of the holding dam.



Careful application of TrzN

**~90% of atrazine removed in less than 4 hrs !!**

Time course of atrazine degradation



Samples analysed by:  
QHFSS (□), and  
CSIRO Entomology (○)

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## Conclusions

- **We have developed an efficient method for producing large quantities of triazine hydrolase using high cell density fermentation**
- **Field trials have established that bioremediation of triazine contaminated water using free triazine hydrolase is technically feasible**
- **Further field trials are underway to assess commercial viability**

# Production of a free-enzyme catalyst for bioremediation using high cell-density fermentation

## Acknowledgements

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Rob Milla



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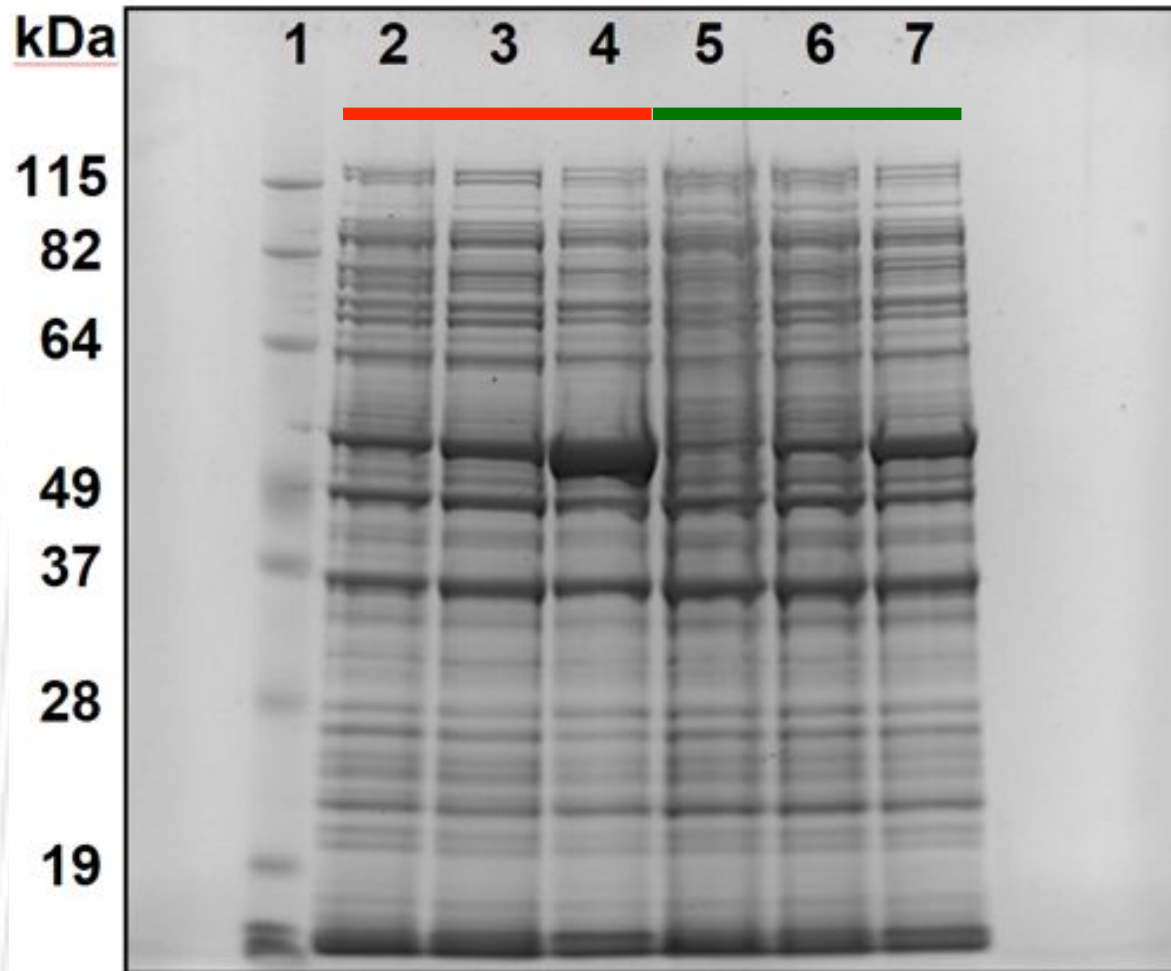
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# Thank You

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# Expression of triazine hydrolase (*trzN*) in *E. coli*



1 – MW markers

**LB – No feed**  
**50  $\mu$ M IPTG induction**

2 – Pre-induction

3 – Induction + 1 hr

4 – Induction + 14.5 hrs

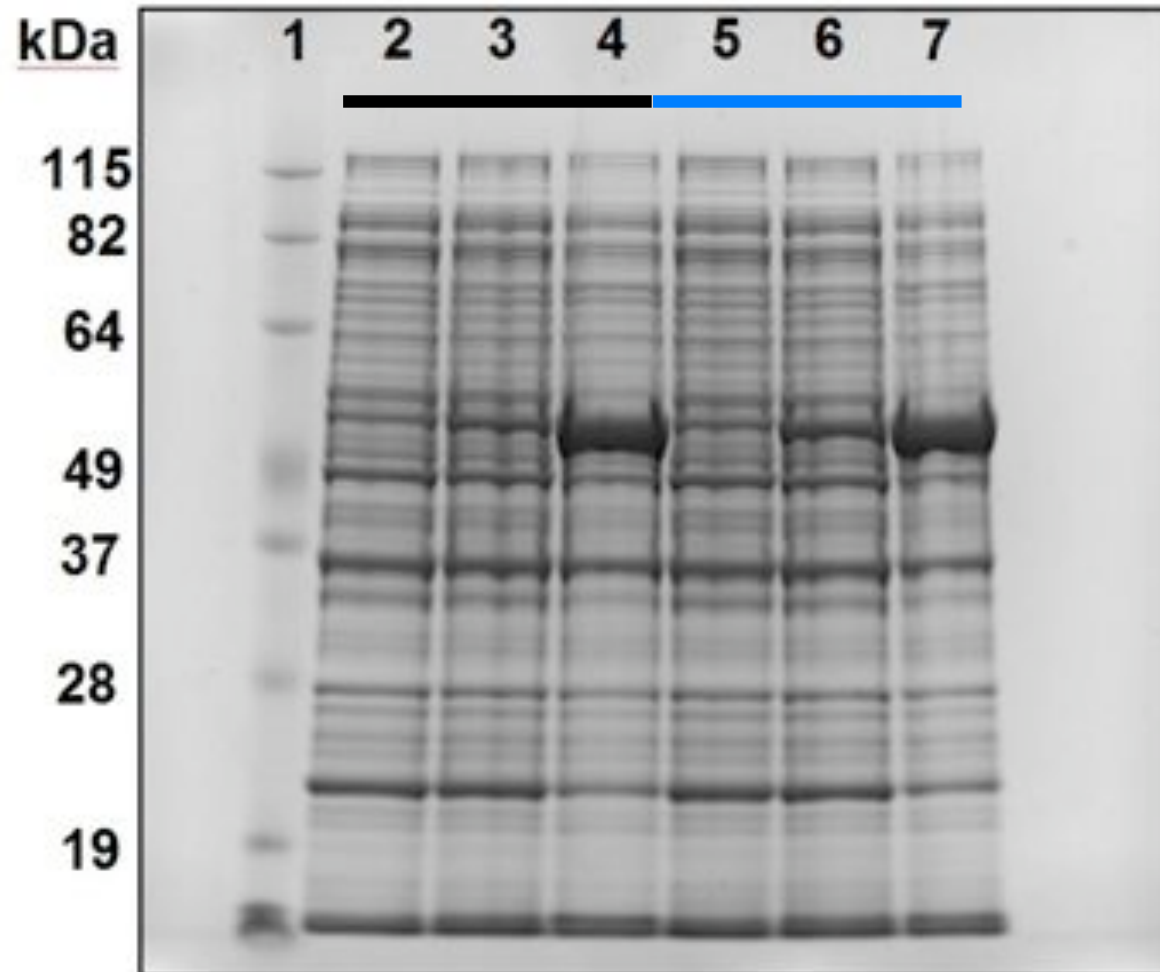
**LB – Glycerol feed**  
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7 – Induction + 14.5 hrs

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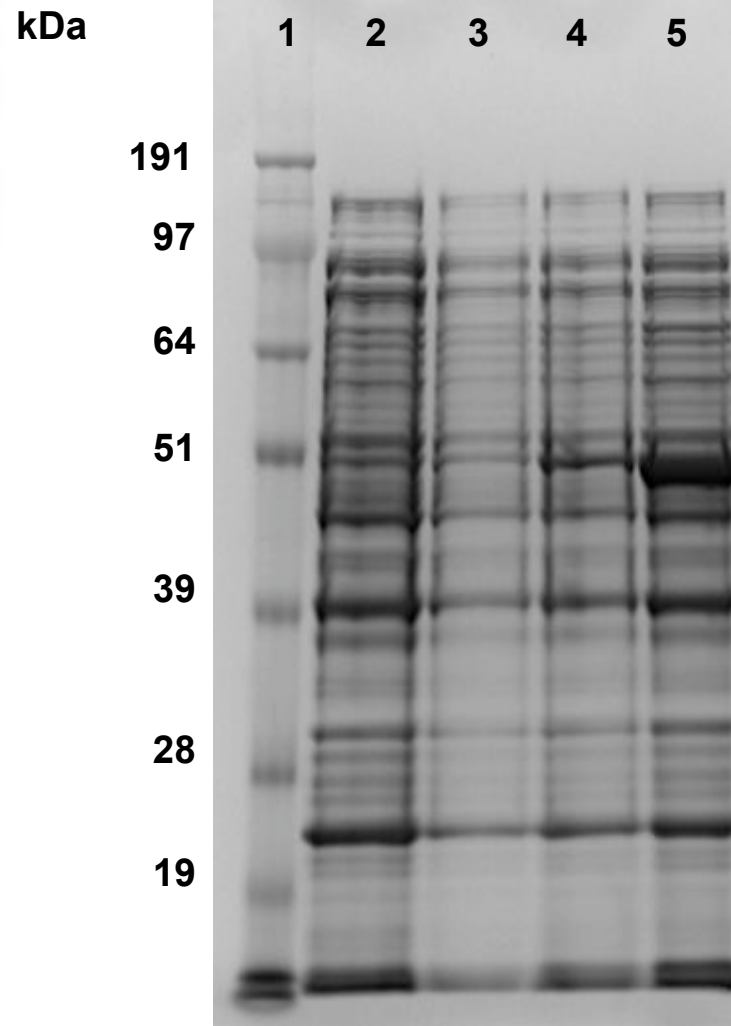
**LB – Glycerol feed**  
**200  $\mu$ M IPTG induction**

5 – Pre-induction

6 – Induction + 1 hr

7 – Induction + 14.5 hrs

# Expression of triazine hydrolase (*trzN*) in *E. coli*



**100-litre fermenter:**

- 1) MW markers**
- 2) Pre-induction**
- 3) Induction + 1 hr**
- 4) Induction + 2 hrs**
- 5) Harvest**

**Pellet weight 14.7 kg**