HERIOT WATT UNIVERSITY

Microbial biosurfactants

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LEADERS IN IDEAS AND SOLUTIONS

Oil & Gas UK the voice of the offshore industry

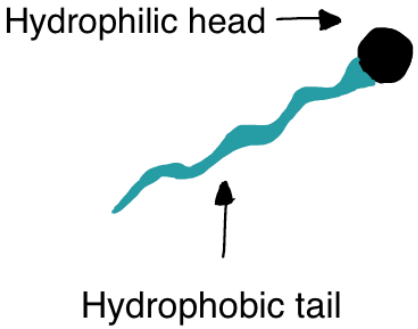
NERC SCIENCE OF THE ENVIRONMENT

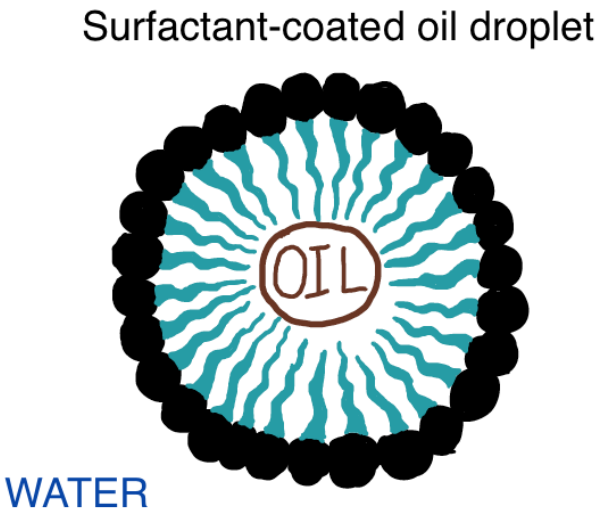
NERC Centre for Doctoral Training in Oil and Gas

A little bit about me

- BSc in Marine Biology (University of Aberdeen). 12 month industrial placement in Shell (Aberdeen).
- NERC CDT in Oil & Gas funded PhD in Heriot-Watt University (Edinburgh).
- **Project: Characterisation and evaluation of microbial biosurfactants effectiveness to disperse crude oil**.
- Research interests: fate and ecotoxicology of hydrocarbons in the marine environment; hydrocarbon-degrading bacteria and their potential for use in the oil and gas industry.

What is a biosurfactant?





- Amphiphilic compounds produced by microorganisms (bacteria, yeast, fungi).
- Reduces surface and interfacial tension between two phases.

Classification

SURFACTANTS

- Low-molecular weight biological molecules
- Comprised of glycolipids, lipopeptides, phospholipids and fatty acids, and lipoproteins
- Tendency to reduce surface tension

EMULSIFIERS

- High-molecular weight biological molecules. Known was exopolysaccharides (EPS).
- More complex in structure – heteropolysaccharides, lipopolysaccharides, lipoproteins and proteins.
- Tendency to emulsify hydrophobic compounds (e.g. oils)

Main producing genera:

Pseudomonas, Bacillius, Alcanivorax, Corynebacterium, Acinetobacter, Rhodococcus, Halomonas, Candida (yeast).

Rosenberg and Ron (1999)

Applications

Advantages:

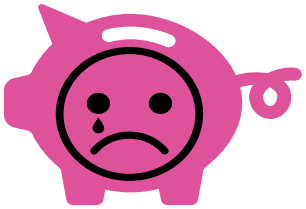
- Natural and biodegradable.
- Non-toxic.
- Requires less energy to produce.
- Industrial waste products as fermentation substrate (e.g. glycerol)
- No unwanted byproducts.
- Safe to use.

Campos et al. (2013); Satpute et al. (2010)

Challenges

- Low production yield – up to 5 g/L.
- Recovery and purification technology at industrial scale still not economical – accounts for up to 80% of the total production cost.
- Time consuming process.
- Best known biosurfactant-producing strains are pathogenic (e.g. *Pseudomonas aeruginosa*).

Market price of rhamnolipid (R-95, 95%) is **£258/10mg** (Sigma-Aldrich)!



Sekhon Randhawa & Rahman (2014)

PhD plan

Isolation and characterization of biosurfactants

- ST and EI
- Stability test
- CMC
- Chemical structural analysis – NMR, HPLC, total amino acids

PHASE 1

Microbial response to biosurfactant and crude oil

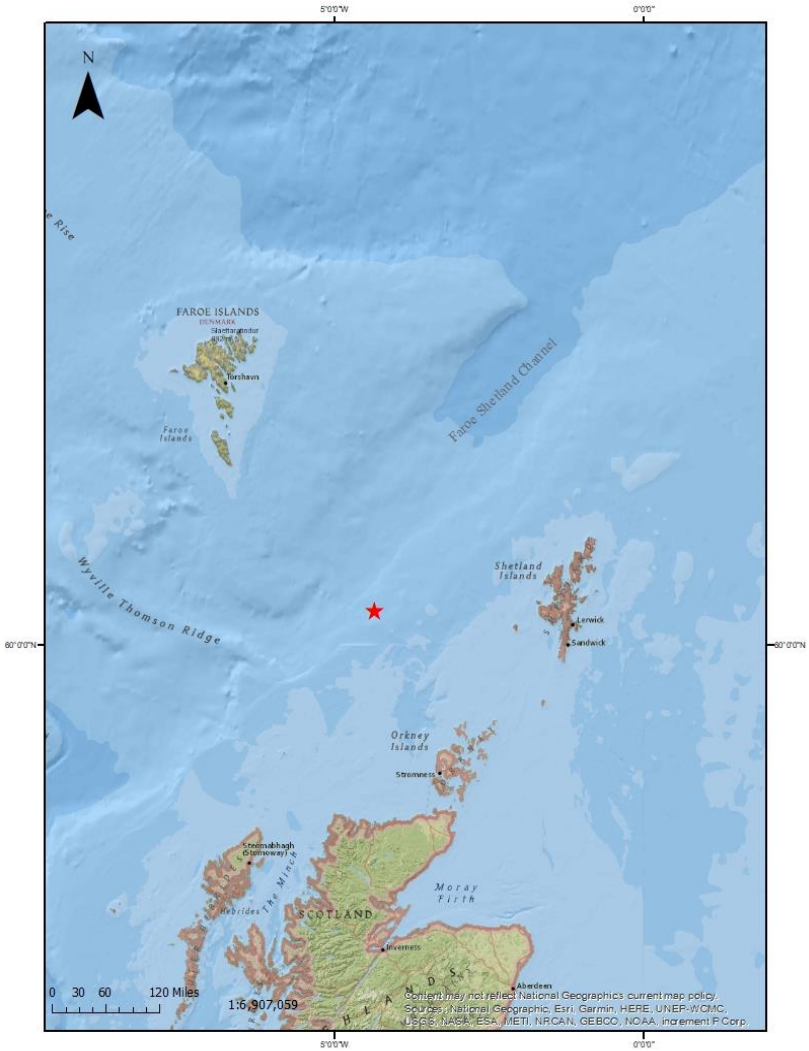
- 4-week lab incubations
- Total cell count
- Next generation sequencing of 16S rRNA gene
- Oil biodegradation (GC-FID/MS)

PHASE 3

PHASE 2

Dispersion efficiency test

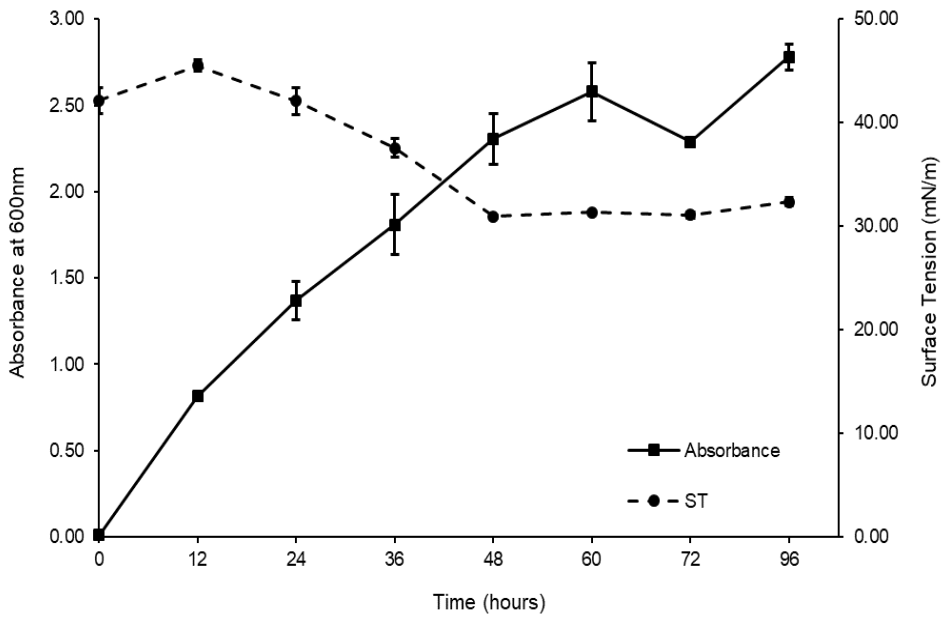
- BOR 1:100; 1:50; 1:20
- Oil-in-water concentration
- Oil droplet size and distribution
- Scheihallion crude



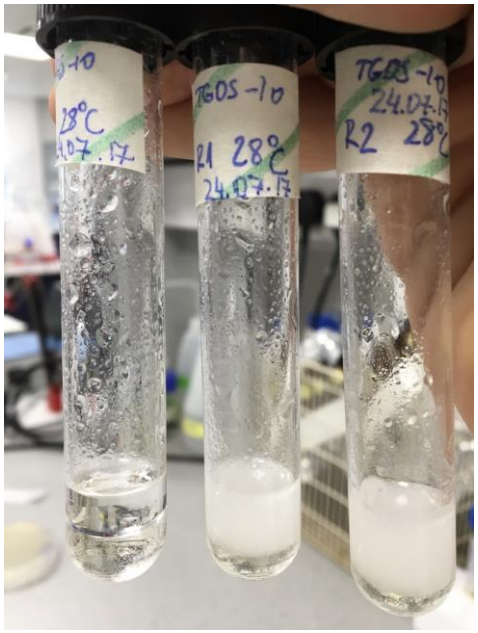
Faroe-Shetland Channel

Bacterial strains of interest

- *Halomonas* sp. strain TGOS-10 – hydrocarbon-degrading; isolated from Gulf of Mexico (Gutierrez et al. 2013).
- Surfactant and emulsifier producer.



Halomonas TGOS-10 at 28C and 150rpm in ZM/1 with 1% v/v sunflower oil and 1% v/v inoculum.

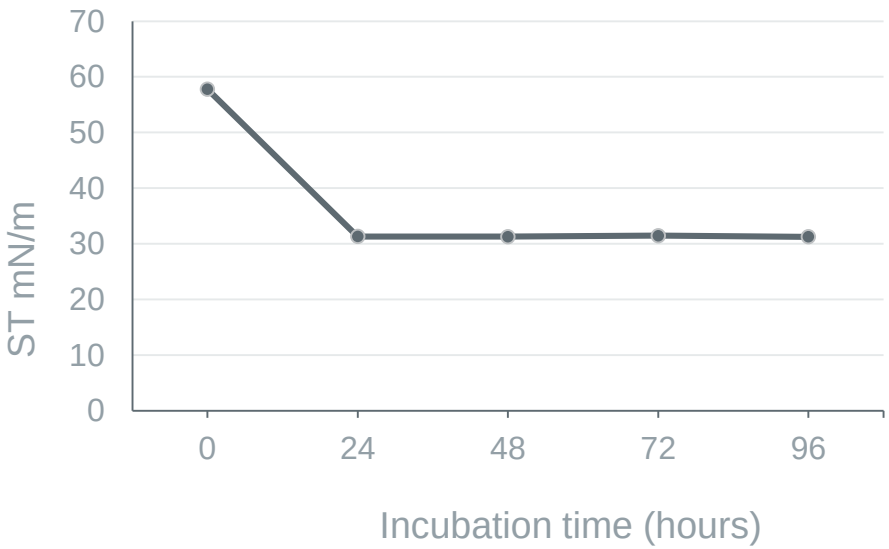


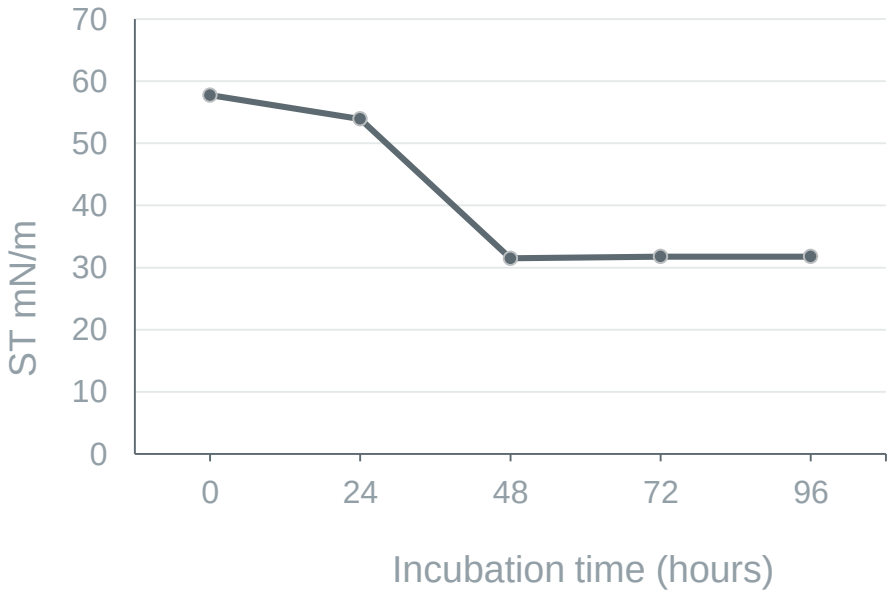
Emulsification Index (EI_{24}) (60%) of hexadecane.

Bacterial strains of interest

- *Pseudomonas** *sp.* strain MCTG214 – hydrocarbon-degrading
- Surfactant producer (rhamnolipid) – Twigg et al. (2018)

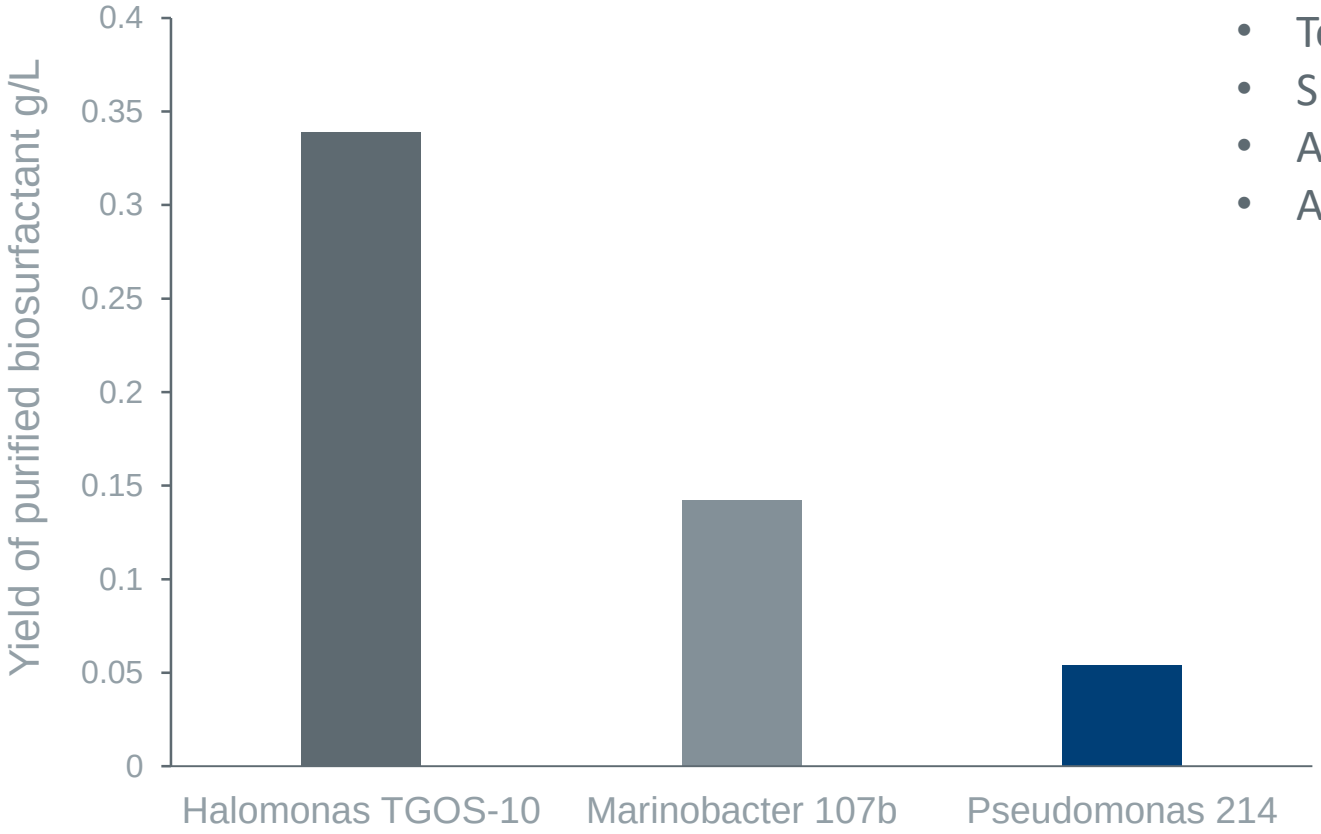
- *Marinobacter sp.* strain MCTG107b – hydrocarbon-degrading
- Surfactant producer (rhamnolipid ?)





* non-pathogenic

Yield of biosurfactant



Depends on:

- Temperature
- Substrate
- Aeration
- Agitation speed

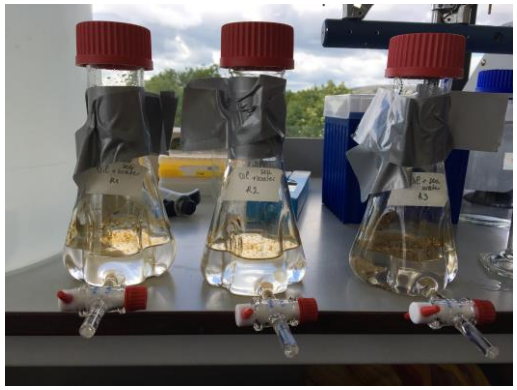
Incubation conditions: 28℃, 150rpm, sunflower oil

Phase 2 – Dispersion efficiency test

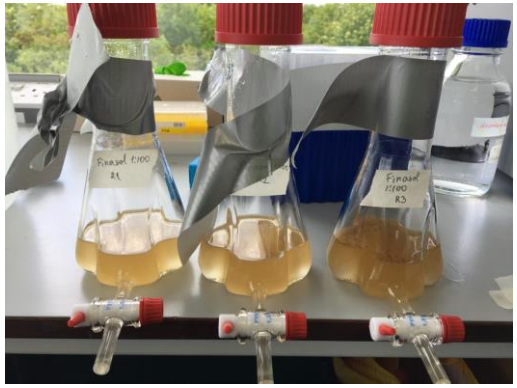
Baffled flask test

- Biosurfactants extracted from *Halomonas* sp. strain TGOS-10 and non-pathogenic *Pseudomonas* sp. strain MCTG 214 (3b1).
- Standard baffled flask test → 10 min shake @ 200rpm; 10 min rest
- Finasol OSR52 and Tween-20 for comparison.
- Dispersant/biosurfactant-to-oil ratio:
- 1:100
- 1:50
- 1:25
- Laser diffraction method to determine dispersion of oil – Turbiscan LAB at Clariant Oil Services (Aberdeen).

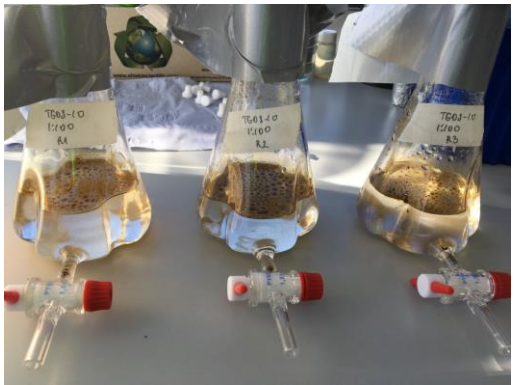
Oil + seawater



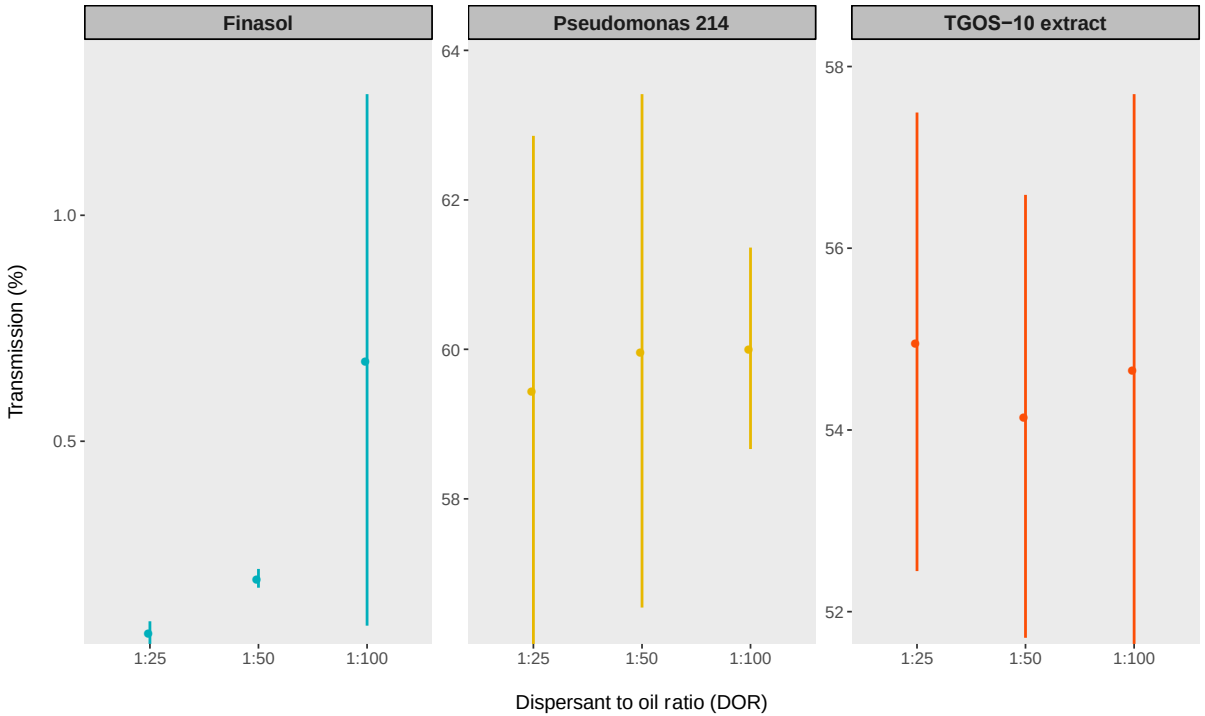
Oil + seawater + Finasol

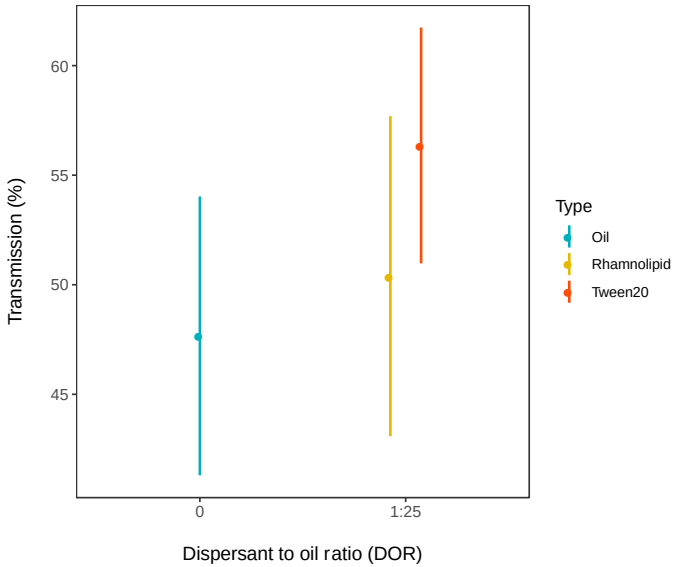


Oil + seawater + TGOS-10 extract



Baffled flask test preliminary results





The lower the transmission %, the higher the concentration of oil in water.

Note: biosurfactants were highly diluted in water prior to test.
Schiehallion crude oil (API 26)

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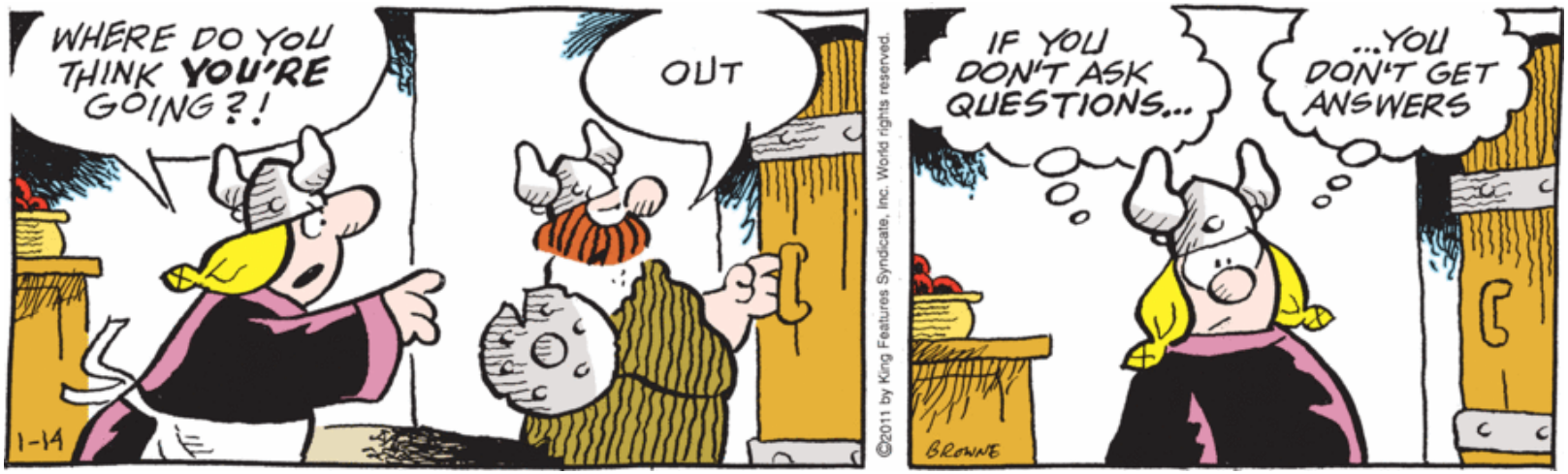
Q&A


WHERE DO YOU THINK YOU'RE GOING?!
OUT
IF YOU DON'T ASK QUESTIONS...
...YOU DON'T GET ANSWERS
1-14
BROWNE
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