9/8/23

	Gen. time	
E. coli	~ 18-20 Mins	more suitable for bioprocess & reactors
V. cholerae	~ 8 mins	
eukaryotes	very high → hours to days.	

1982 [Pseudomonas putida - aromatic hydrocarbons
E. coli - recombinant insulin

Post-translational modifications → not present in E. coli cells. Only small proteins such as insulin could be produced.

Yeast (S. cerevisiae) → alternative host (eukaryotic)

but yeast was quarantining these recombinant proteins & were not secreting these proteins

Pichia pastoris (another yeast) → did not quarantine the proteins

Interleukins, Interferons → Cytokines

Animal cell lines → CHO - Chinese Hamster Ovary
BHK - Baby Hamster Kidney

Amgen → Erythropoietin → 1990s
to solve cancer-related anaemia

Multiple gene Multiple products → Metabolic Engineering → Systems Biology & Synthetic Biology

16/8/23

Starting material < whole cells, enzymes → Biomass

Bioprocess ↓

Product → Biofuels

Bioprocessing { Upstream processing → Midstream processing → Downstream processing

Somatostatin → 1970s → not recombinant

↓ used for lowering blood cholesterol

United Nations Framework Convention on Climate Change → Paris, 2015.

- Anthropogenic activities → CO_2, CH_4
- By 2050 : energy demand will double or even treble.
- 2008 : thought to be the peak of global petroleum prod^n, but now it is believed that the peak is yet to come.

→ IPCC report on Climate Change, 2007

OPEC countries: Organization of Petroleum Enhanced Countries.

MEOR: Microbially Enhanced Oil Recovery.

Need for EOR:

- ✓ oil reservoir → drilled
- ✓ oil recovered → 30-50%
- ✓ oil left → 50-70% } EOR target

production well → drill → release the pressure → natural drive of oil (10-15%) — primary recovery

once natural pressure is extinguished → suction → Heavy Duty Centrifugal Pumps (another 10-20%) — secondary recovery → EOR

--- → water flooding → water jet + EOR agents

but oil is immiscible in water, so not much oil comes out

EOR agents
- chemical surfactants
- Polymers
- Solvents
- Acids

Tertiary recovery / EOR (another 10-15%)

surfactants → reduce the surface tension of oil

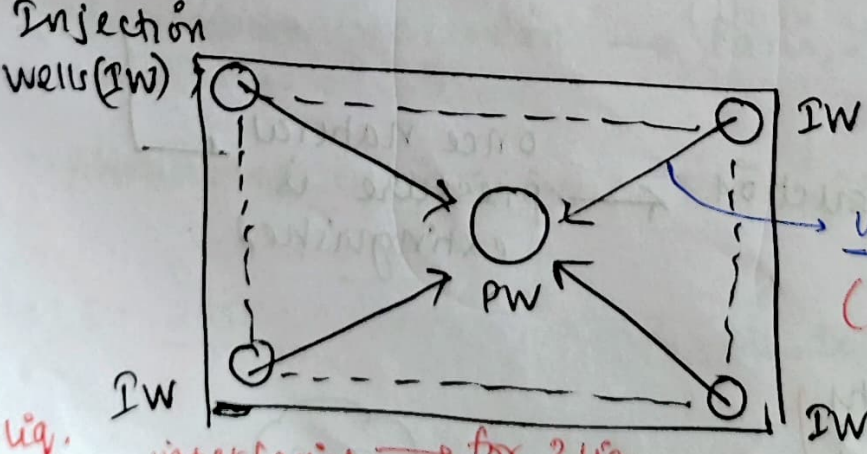
Polymers → viscosify / thicken water → push oil more effectively

After these 3 steps → ~40% of oil is recovered.

↓

after this, the oil company loses interest & goes to a different place

OOIP - original oil in place → data from the Geological Survey of India (GSI)



interfacial tension pure → for 2 liq.

ST / IFT of water = 72 dyne/cm, 72 mN/m → reduced to ~30 by EOR agents.

for 1 liq. ↑ surface tension

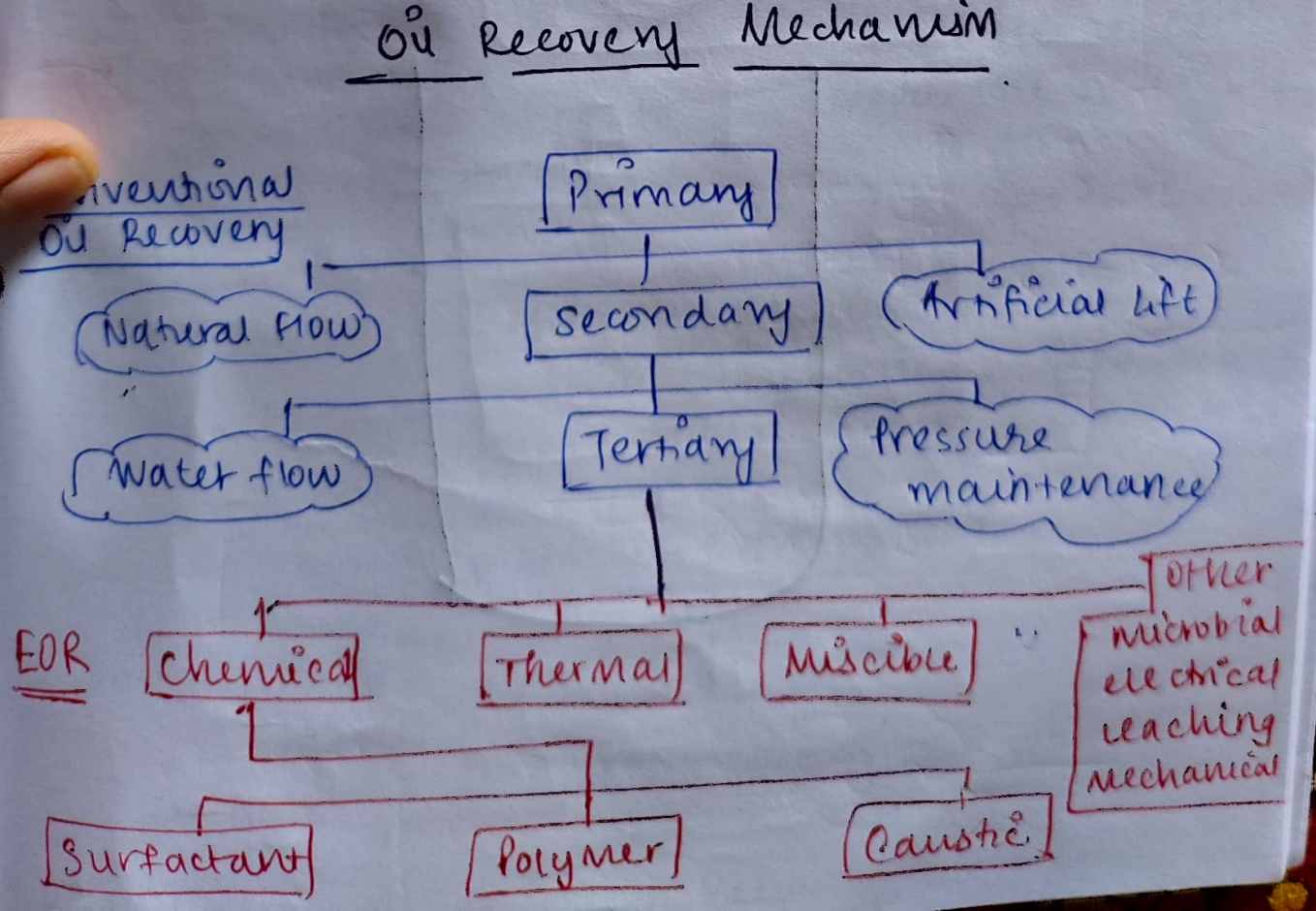
- But EOR agents → are themselves produced from petroleum
  ↓
  alternative?
  ↓
  MEOR!

23/8/23

> 90% of water jet comes out w/o oil → Water cut

targets of MEOR ← microorganisms
enzymes
GMO (but risky)

Oil Recovery Mechanism

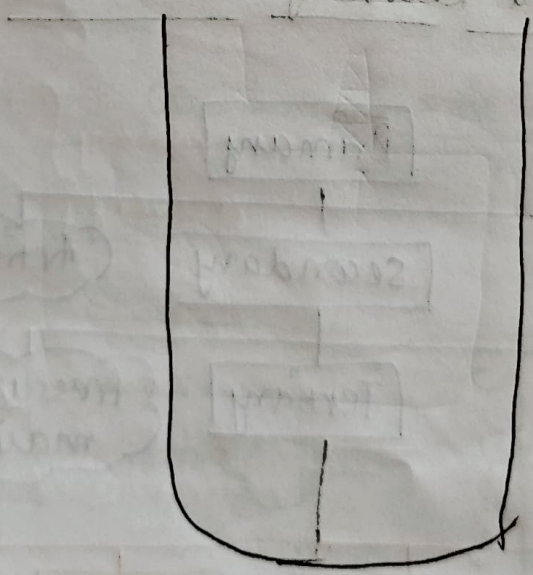


2nd & 3rd gen. EOR methods

- Water flooding (water injection)
- Gas injection (no miscible)
- Carbon dioxide flooding (miscible)
- Steam injection & in-situ burning
- Surfactants or foams injection including Alkali - surfactant injection

LABS → linear alkene benzene sulfonates

- Microbial Enhanced Oil Recovery
  - biosurfactants
  - emulsifiers-
  - biopolymers



What is MEOR?

- use of microbes
- improve oil recovery
- Beckman - 1926
- 60% OOIP after I recovery

1

Ex-situ

produced outside, in some factories

↓

brought to the oil fields: the microbial products

In-situ

consortium of bacteria producing surfactant, polymers, etc.

+

nutrients

↓

supplying the broth to the reservoir ~~by~~ via the IWs.

↓

close the oil wells & allow the microbes to grow

← train them to feed on oil & grow

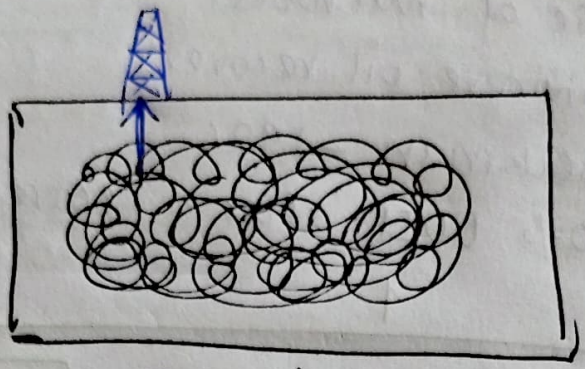
↳ they should not grow on the shorter-chain hydrocarbons → feed on long chain HCs (C>20).

as these are our useful products

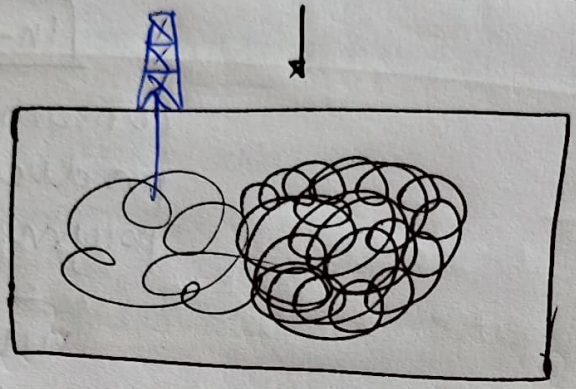
- thermophilic
- osmophilic
- barophilic
- alkaliphilic

"Biotechnology in Petroleum Recovery: The microbial EOR"

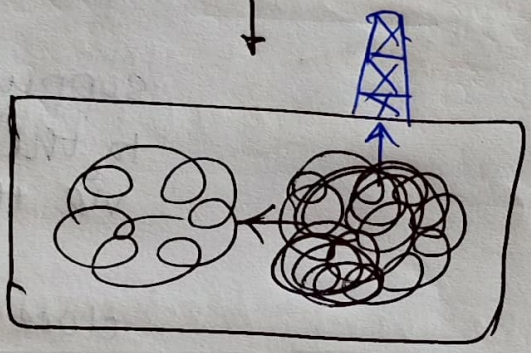
oil rig is set up at an oil-rich place



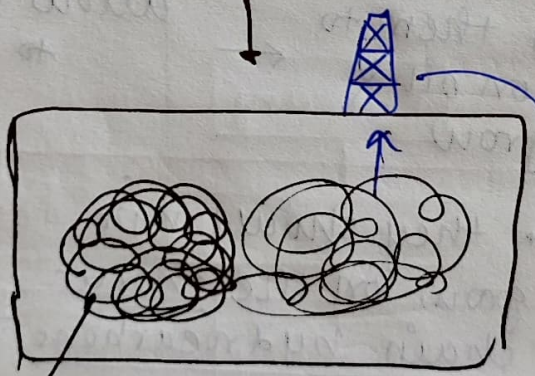
eventually this place becomes oil-less



by simple diffusion the oil from the oil-rich zone comes ~~to~~ to the oil-less zone



the companies try to shift to the oil-rich zone adjacent but the previously oil-less zone is 'thieving' on new zones



not much production

Thief zones

Thief zones need to be blocked

- Thermosetting Polymers
- Biomass (MEOR)

30/8/23

derived from plants & microbes

Biosurfactants

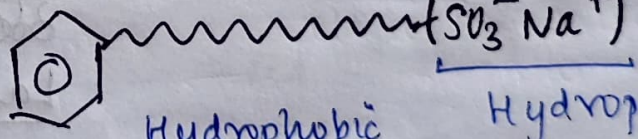
- reduces surface tension (ST) / interfacial tension (IFT) (mN/m) or (dynes/cm)
  - b/w liquid & air
  - b/w two liquids
    - in MEOR, this is reduced between ~~petro~~ water & crude oil
- causes emulsification
- Hydrophobic & Hydrophilic (Lipophilic) moieties/domains in the same molecular structure (Lipophobic)

commercial detergents → LAB's: linear alkyl benzene sulphonates

Soap: HC tail (hydrophobic) — $(COO^- Na^+)$ (hydrophilic)

fatty acid

Detergents:



Hydrophobic — Hydrophilic

Amphipathic / Amphiphilic

Hydrophilic + Hydrophobic

Saponin: plant-based surfactant (powerful)

Biosurfactants:

- Degradable
- less harmful than chemical surfactants
- Green surfactants

Oleochemical surfactants

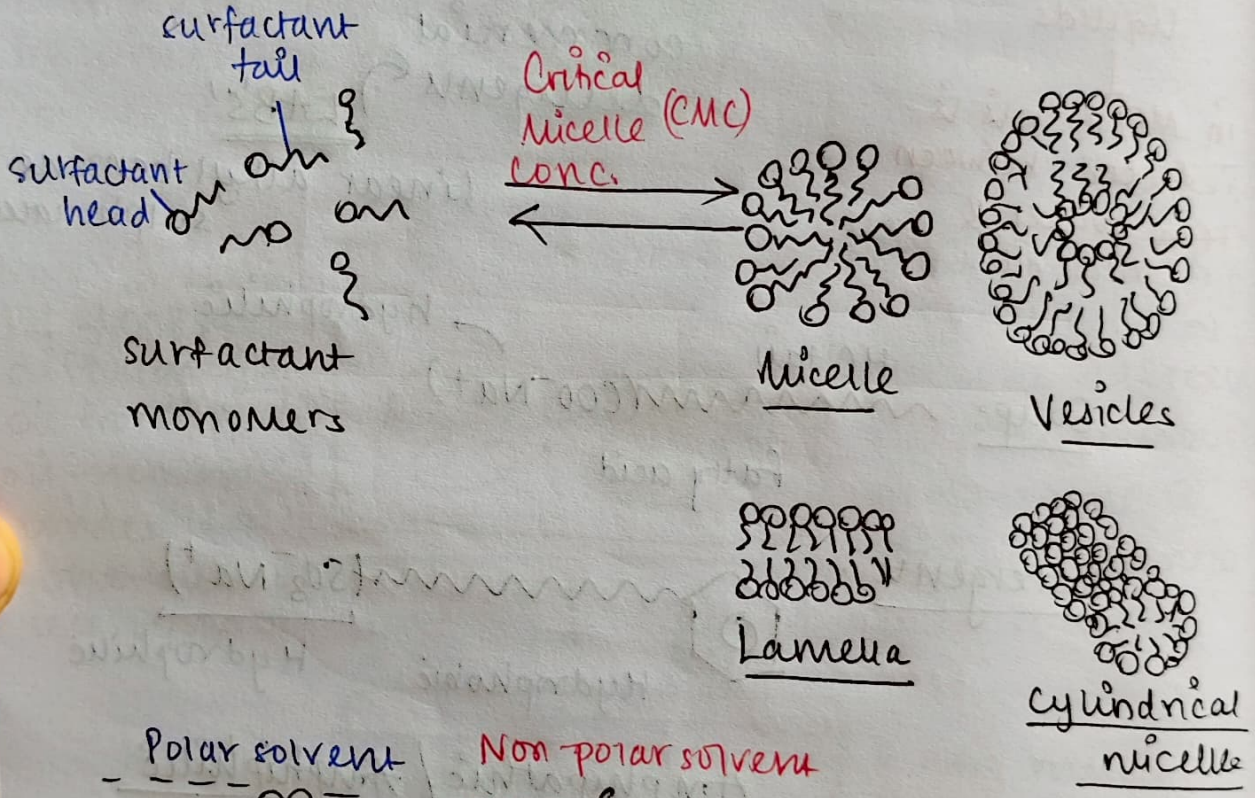
↳ derived from plant-based/ vegetables-derived oils. (edible & non-edible oils)

eg: SDS

Biosurfactants:

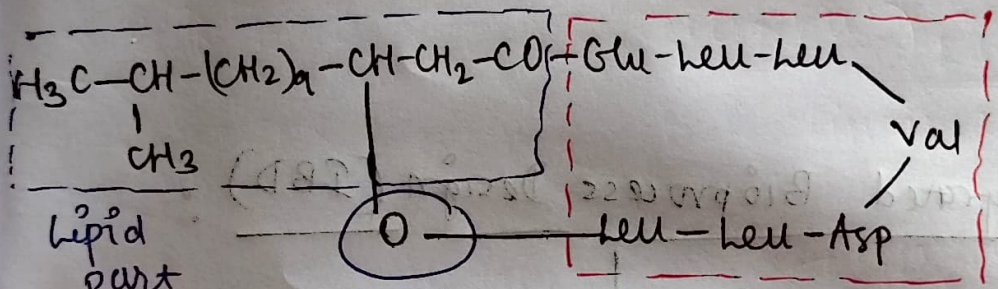
↳ mostly enzymatically-synthesized

eg: AGPs, sphorolipids



Biosurfactants

- **Glycolipids**
  - Rhamnolipids
  - Sophorolipids
  - Trehalolipids
  - sugars → *P. aeruginosa*
- **Lipopeptides / Lipoproteins**
  - Surfactin
  - Neutral lipids
- **Fatty acids**
  - Polymeric biosurfactants
- **Phospholipids**
  - Particulate biosurfactants



Surfactin → *B. subtilis*

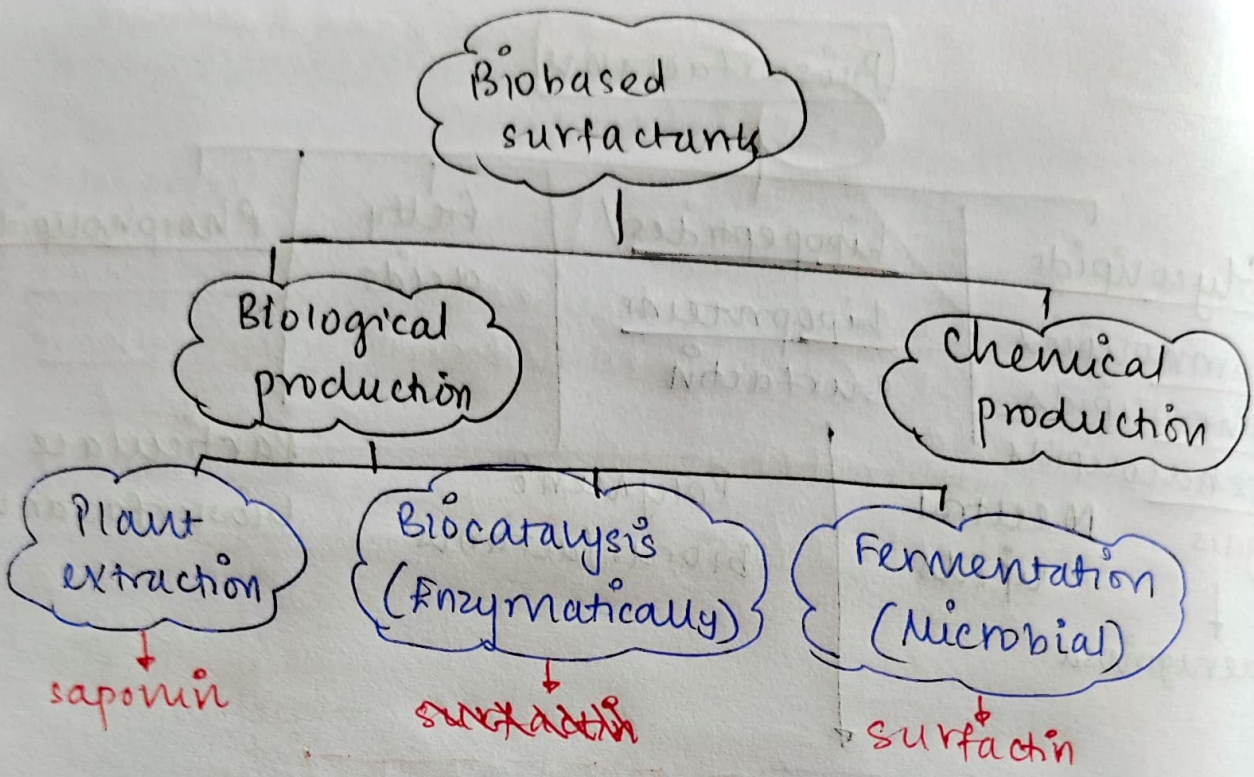
Nanoparticle formation → we need **reducing agent**

eg: $Ag^+ \rightarrow Ag^0$ → metallic silver nanoparticles

eg: sodium borohydrate biosurfactants → not only help in reducing, but also protect them

↓

Capping agents → prevent them from forming aggregates ← stabilize them as individual nanoparticles



Integrated Bioprocess Design (IBD):

- Host selection
  - Metabolic capability
- Pathway optimizn
- Process optimizn

Every scale-up step:

- P/V constant
- Impeller Re No. const
- mass transfer coeff.

6/9/23

PNI diagram : Process & Instrumentation Diagram

↓

Batch Process Simul^{n} & Modelling

Protein purification

↳ precipitation

↳ Ammonium sulphate

- solvent extr^{n}
- salt extr^{n} (salting out)

↳ hydrophobic a.a. residues are exposed in water → thrown out of the medium → precipitation of proteins

→ Contaminants in Rhamnolipid production

If process is asked → draw the diagram

↓

refer the papers of PNI diagram

↳ Upstream processing
↳ Midstream "
↳ Downstream "

Green Polymers

Biopolymers

eg: Dextran

Sources [Plants, Animals, Microbial

Synthetic polymers: → petroleum-based polymers
- non-biodegradable
- non-biocompatible

Biopolymer Types

long chain carbohydrate polymers → can be separated by acetone
- Levan family
- Curdlan family
- Geran family

emulsifiers: 72 mN/m → 45 mN/m } ST of H_2O

surfactants: 72 mN/m → <30 mN/m } ST of H_2O

Bacillus licheniformis → from hot springs of Bakreshwar

→ Thermophilic bacteria

↓

some even Archae