The use of immobilized microbial cells for wastewater treatment

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Review Questions

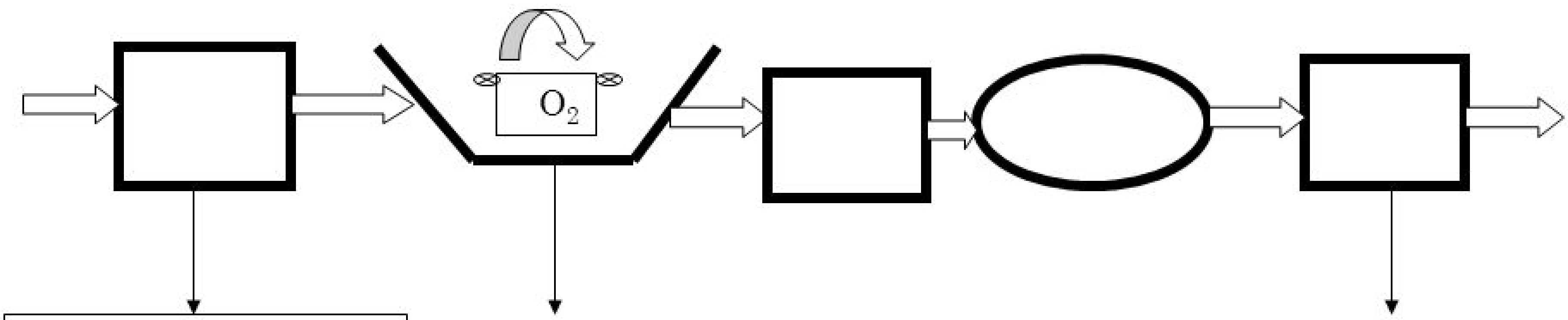
- What is the **immobilization**?
- What are the **methods** of immobilization?
- What are the Industrial **applications** of immobilized cells?

- What is **Wastewater Treatment**?

What is Wastewater Treatment?

- *Wastewater treatment* is the process of converting wastewater – water that is no longer needed or is no longer suitable for use – into water that can be discharged back into the environment.
- It's *formed by* a number of activities including bathing, washing, using the toilet, and rainwater runoff.
- Wastewater is *full of contaminants* including bacteria, chemicals and other toxins.
- Its treatment *aims at reducing* the contaminants to acceptable levels to make the water **safe** for discharge back into the environment.

Wastewater Treatment Processes



Primary treatment
- screening
- grit removal
- removal of oil
- sedimentation

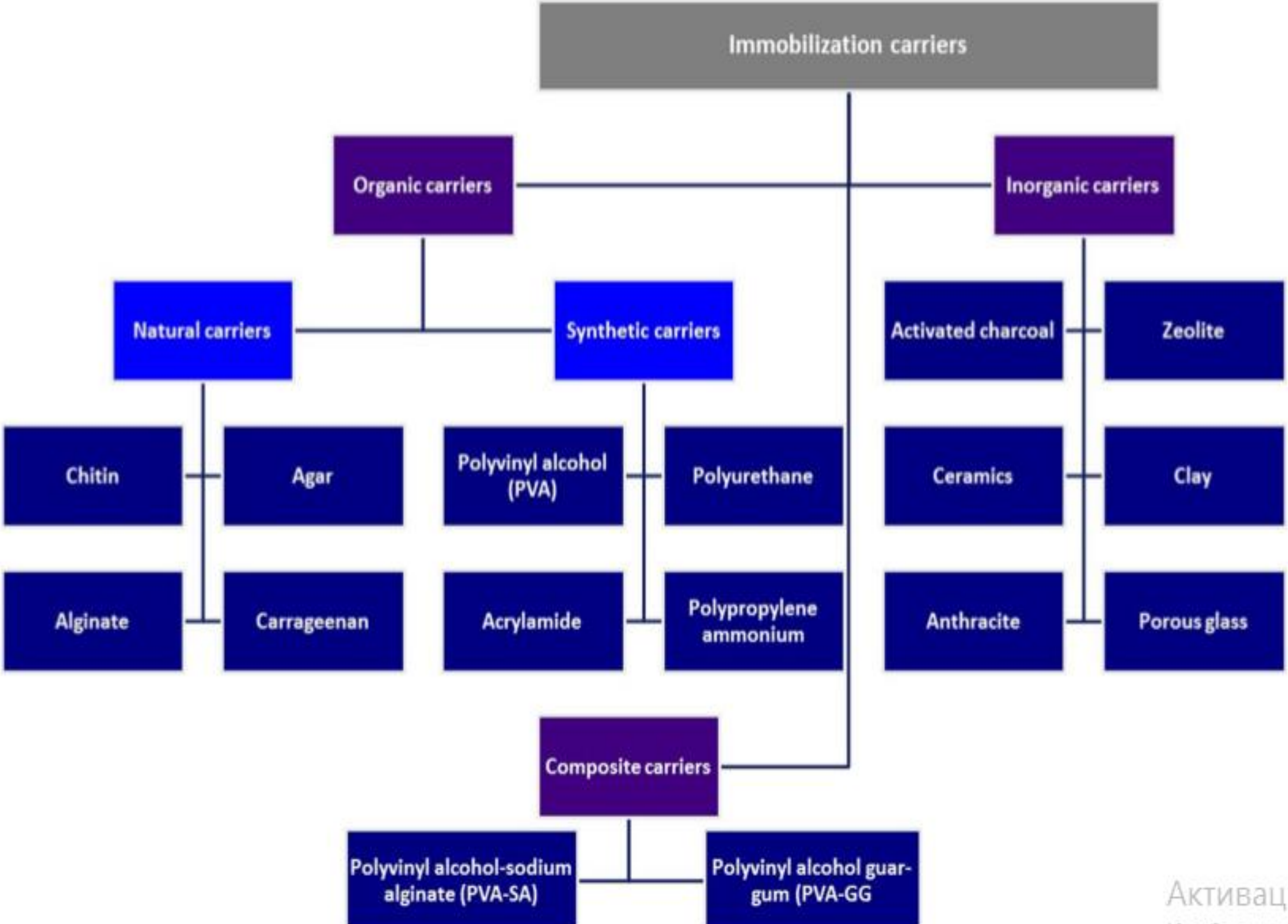
Secondary treatment
- Aerobic, anaerobic lagoons
- Trickling filter- activated sludge-oxidation ditch
- Mostly BOD removal technology

Tertiary treatment
- Nitrate removal
- Phosphorus removal
- Disinfection

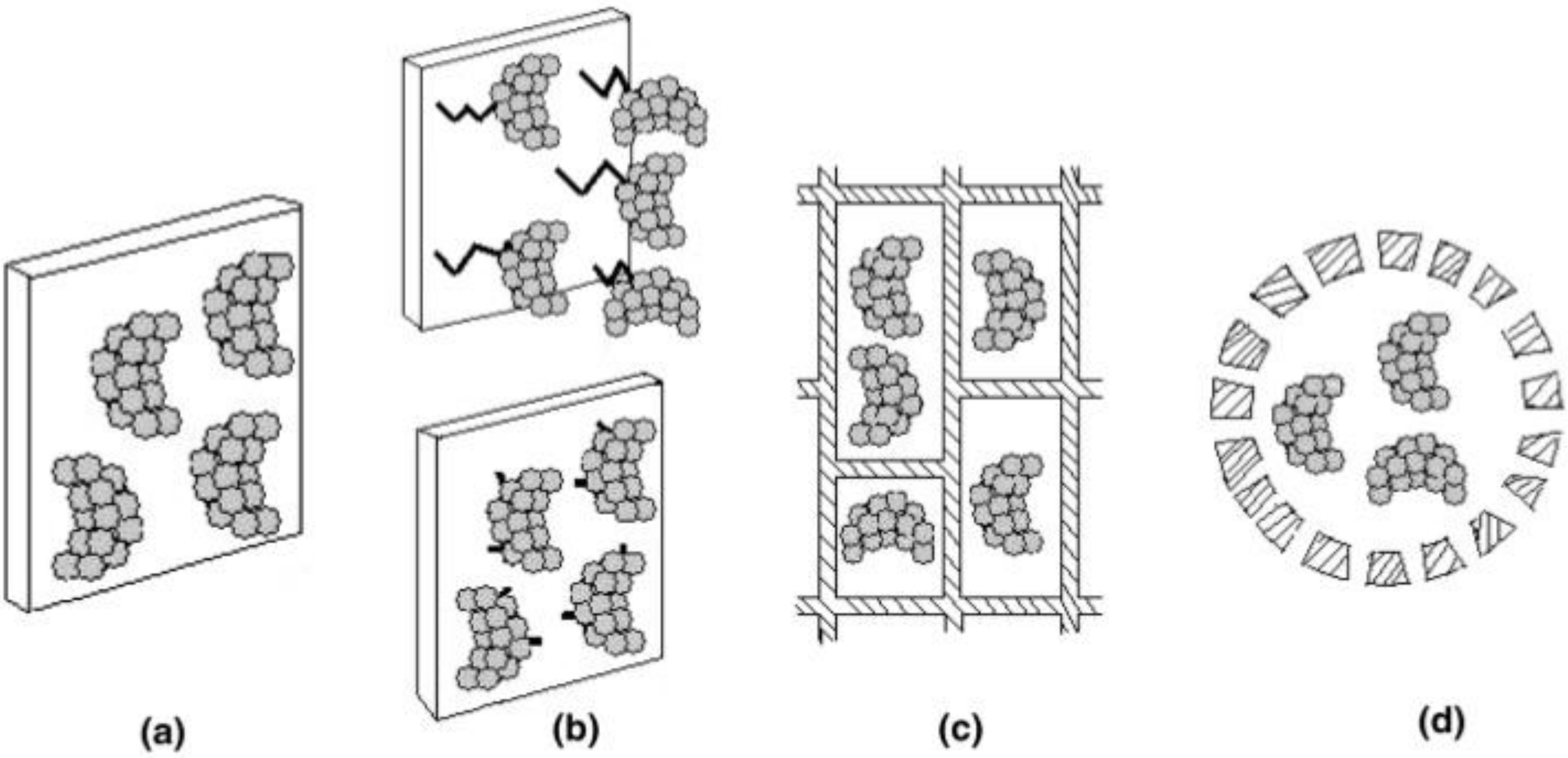
- There are several methods for treating industrial wastewater such as *mechanical, biological and chemical* methods.
- ***Biotechnological processes***, which involve utilizing microorganisms with the objective of degrading wastewater contaminants, have been widely applied for wastewater treatment purposes.
- *Researches proved that biological treatment strategy is cost-effective, which does not require the addition of any dangerous chemicals and helps in a complete destruction of biodegradable pollutants.*
- *On the other hand, employing microorganisms in the removal of contaminants from wastewater is often associated with the problem of their separation difficulty after treatment.*

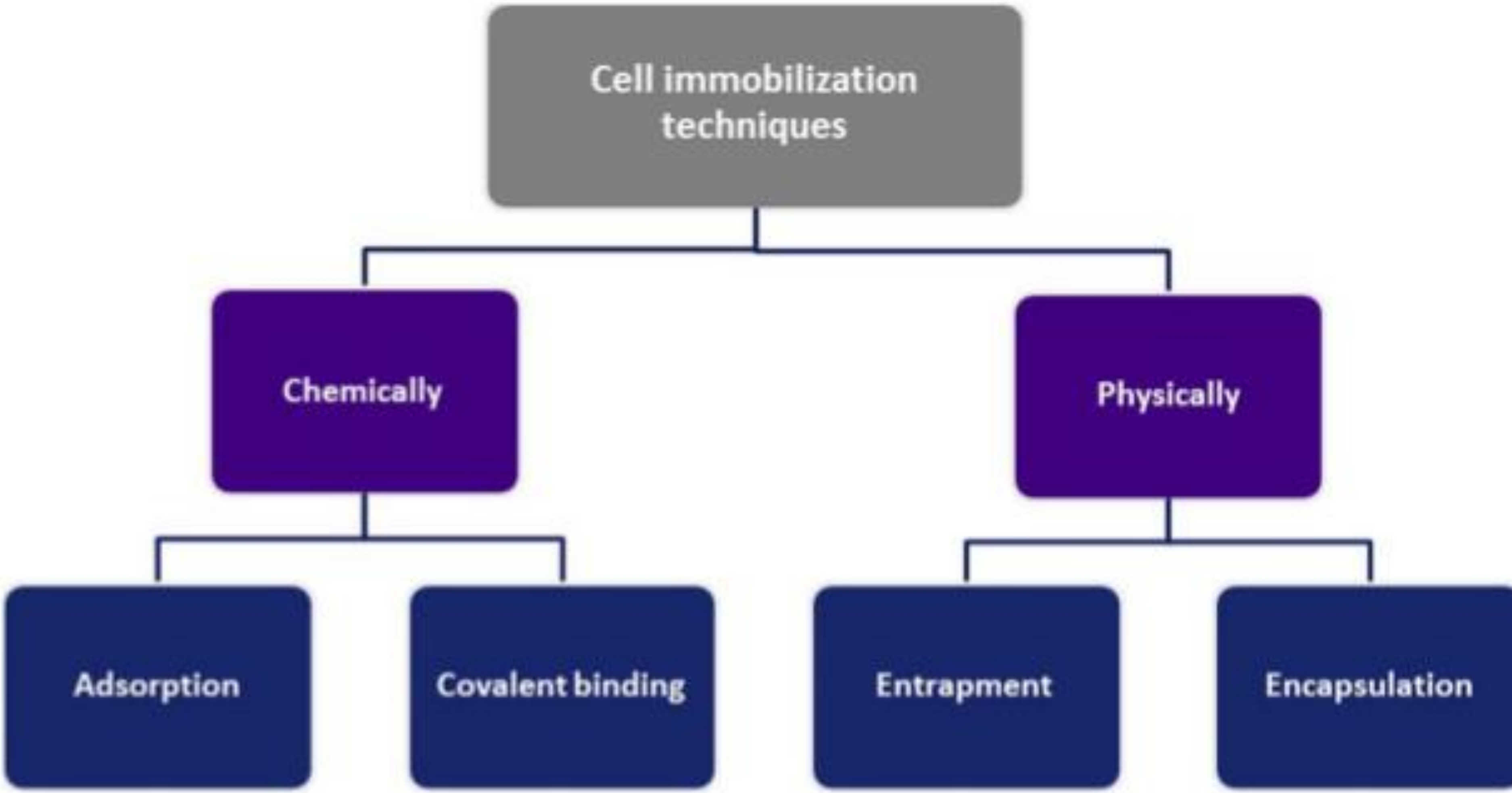
- Industrial application of biotechnological strategies is limited by two main factors: ***the lack of long-term operational stability*** and ***the difficult recovery and reuse of the cells***.
- Therefore, cell immobilization, which represents the restriction of cell mobility by chemical or physical means, can overcome these drawbacks.
- The application of cell immobilization technology in the field of wastewater treatment is gaining importance in that it provides several advantages compared with biodegradation using free cells, such as *providing high biomass, cell reuse, high mechanical strength, high resistance to toxic chemicals, improving genetic stability and eliminating cell washout problems*

- The following immobilized systems were employed for different types of wastewater and have shown the ability to a high extent degradation:
- microalga (*Isochrysis galbana*) biomass was immobilized via entrapment into alginate gel,
- ammonia-oxidizing bacteria were immobilized into calcium alginate matrix, and
- *Pseudomonas putida* was immobilized in polyvinyl alcohol (PVA) particles.


Immobilization carriers
Organic carriers
Inorganic carriers
Natural carriers
Synthetic carriers
Activated charcoal
Zeolite
Chitin
Agar
Polyvinyl alcohol (PVA)
Polyurethane
Ceramics
Clay
Alginate
Carrageenan
Acrylamide
Polypropylene ammonium
Anthracite
Porous glass
Composite carriers
Polyvinyl alcohol-sodium alginate (PVA-SA)
Polyvinyl alcohol guar-gum (PVA-GG

- Different cell immobilization methods have been applied to bind microorganisms on carriers using artificial ways




Cell immobilization techniques
Chemically
Physically
Adsorption
Covalent binding
Entrapment
Encapsulation

1. Treatment of refractory organics wastewater
2. Treatment of wastewater containing heavy metal ions
3. Treatment of nitrogen and phosphorus in wastewater
4. Decolonization of industrial dyes

Treatment of refractory organics wastewater

- *Refractory organic compounds* are known to be difficult to biodegrade and exhibit a low value for the biological oxygen demand to chemical oxygen demand ratio
- Refractory organic compounds, such as phenol, cyanide and aniline, are not efficiently degraded via conventional biological wastewater treatment
- This is due to the long period required for the growth of microorganisms; they are also difficult to exist in biological treatment structures.

Microorganism	Degraded material	Immobilizing matrix
Pseudomonas putida	Phenol	Polyvinyl alcohol (PVA) gel
Bacillus cells	Phenol	Polyvinyl alcohol-sodium alginate (PVA-SA)
		Polyvinyl alcohol-guar gum (PVA-GG)
Klebsiella oxytoca	KCN (Cyanide)	Alginate
		cellulose triacetate
Pseudomonas putida	Phenol	Polyvinyl alcohol (PVA)
Pseudomonas putida	Phenol	Polyvinyl alcohol (PVA)
Bacillus species	Phenol	Tea waste biomass
Pseudomonas fluorescens	Ferrous(II) cyanides complex	Calcium alginate
Pseudomonas putida	Phenol	Alginate
Flavobacterium species	Pentachlorophenol	Polyurethane
Ralstonia pickettii	Phenol	Polyvinyl alcohol (PVA)
Acinetobacter species *strain* PD12	Phenol	Polyvinyl alcohol (PVA)
methanogenic consortium	Phenol	Agar
Pseudomonas putida BCRc14349	Phenol, trichloroethane	Chitosan
Strain XA05 and FG03	Phenol	Polyvinyl alcohol (PVA)
Pseudomonas putida	Phenol	Calcium Alginate
Candida species and *Pseudomonas* species	Phenol	Activated carbon
Pseudomonas putida	Cyanides, cyanates, and thiocyanates	(Agar, alginate or carrageenan)
Pseudomonas putida	Phenol *o*-, *p*- and *m*-cresols	Polyvinyl alcohol (PVA)
Pseudomonas putida	*p*-cresol	Polyvinyl alcohol (PVA)

Treatment of wastewater containing heavy metal ions

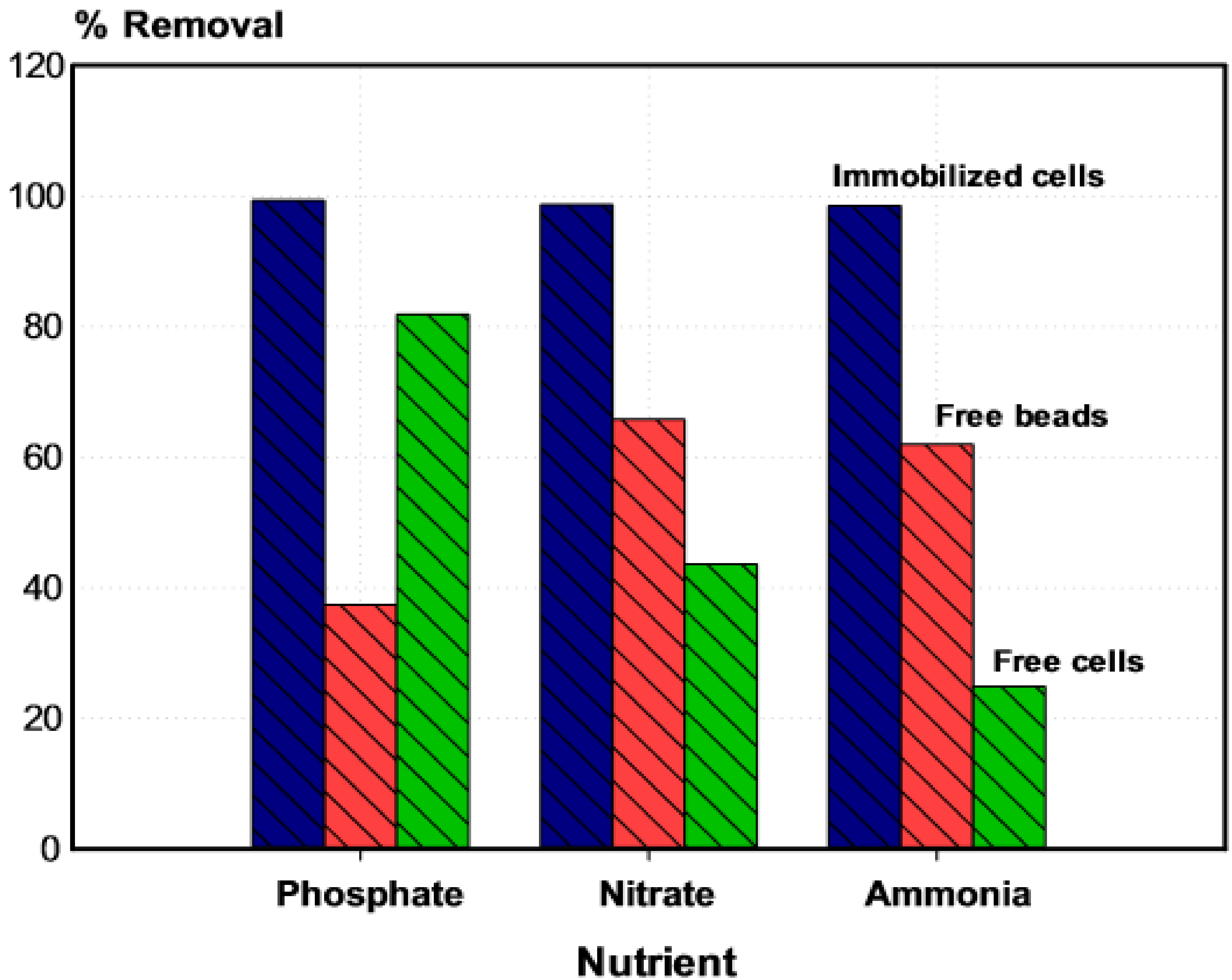
- Most types of industrial wastewater contain considerable amounts of ***toxic metal ions*** such as cadmium, mercury, lead and copper, which can cause serious health concerns by producing ***free radicals***.
- Among all types of microorganisms used for bioremediation purpose, ***microalgae*** provide a high capacity to remove heavy metal.

Microorganism	Removed metal	Immobilizing matrix
Chlorella	Ni, Zn, Cd	Alginate
Chlorella vulgaris	Cu, Ni	Alginate
Chlorella vulgaris Scenedesmus acutus	Zn, Cd, Cr	Carrageenan beads
		Polyurethane foam
Chlorella emersonii	Mercury	Agarose beads
		Agar beads
		Alginate beads
Chlorella species	Uranium	Polyacrylamide gels
Stichococcus bacillaris	Lead	Silica gel
Pilayella littoralis		
Chlamydomonas reinhardtii	Uranium	Carboxymethyl cellulose (CMC) beads
Chlorella vulgaris	Hg	Silica gel
Tetraselmis chui	Cu, Cd	Alginate
Aulosira fertilissima	Ni, Cr	Glass beads
Penicillium citrinum	Cu(II)	Calcium alginate
Bacillus subtilis	Cu(II)	Chitosan
Microalga (*Isochrysis galbana*)	Cr(III)	Alginate gel
Algal cells (*Pediastrum boryanum*)	Cr(VI)	Alginate
		Alginate–gelatin
Bacterial strain (*Corynebacterium glutamicum*)	As(III), As(V)	Neem leaves/$MnFe_2O_4$ composite
Bacillus species	Cr(VI)	Tea waste biomass
Modified sludge biomass (MSB)	Cu(II) Cr(VI)	Calcium Alginate
		Polyvinyl alcohol (PVA)

Treatment of nitrogen and phosphorus in wastewater

- Nutrients (NH_4^+, NO_3^- and PO_4^{-3}) are considered the main factors leading to eutrophication in natural waters when wastewater containing nutrients run into lakes and rivers
- Megharaj el al. (1992) investigated the efficiency of two types of soil microalgae, *Chlorella Vulgaris*, and *Scenedesmus bijugatus*, to remove ammoniacal nitrogen. Cells of the microalgae were immobilized by entrapment into calcium alginate beads. Result: 71–79% of NH_4–N was removed.

- Dong et al. (2014) studied the removal of ammonia by *Ammonia-oxidizing* bacteria immobilized on sodium alginate. Ammonia removal rate of 89.51% was achieved.
- Another study was conducted by Jaysudha and Sampathkumar (2014) to examine the removal of phosphate, nitrate and ammonia using free and immobilized marine microalgae, *Chlorella salina*, while immobilization was carried out in sodium alginate matrix.
- It was found that immobilized cells absorbed 99.39% of phosphate, whereas free beads and free algal cells, respectively, removed only 37.41% and 81.94% of phosphate. Likewise, 98.72, 65.83 and 43.71% of nitrate were removed by immobilized cells, free algal cells and without microalgal beads, respectively. Finally, 98.54% of ammonia was absorbed by immobilized cells when compared to free algal cells and algae-free beads in 62.04% and 24.94%, respectively.



% Removal
120
100
80
60
40
20
0
Immobilized cells
Free beads
Free cells
Phosphate
Nitrate
Ammonia
Nutrient

Microorganism	Application	Immobilizing matrix
Photosynthetic Bacteria	Nitrates	Na-Alginate
		PVA
Microalgae	Nutrients	Agar–alginate algal blocks (A.
Nitrite-oxidizing bacteria (NOB)	Nitrite	Biopolymeric chitosan
Ammonia-oxidizing bacteria	Ammonium	Sodium alginate
Nitrifier cells	NH_4-N	Polyvinyl alcohol and sodium alginate
Chlorella vulgaris Azospirillum brasilense	Ammonium and phosphorous ions	Alginate
Scenedesmus intermedius	Phosphorus and nitrogen	Alginate
Scenedesmus species	Ammonia–nitrogen and Orthophosphate	Alginate
Green microalga (*Chlorella vulgaris*)	Ammonium, phosphate	Calcium alginate
Algal (*Chlorella vulgaris* and *Azospirillum brasilense*)	Ammonium, phosphate	Alginate
Spirulina maxima	Phosphorus, ammonium	Carrageenan beads
Chlorella vulgaris; cyanobacterium Anabaena doliolum	Phosphate, nitrate, nitrite	Agar beads
		Alginate beads
		Carrageenan beads
		Chitosan beads

Decolonization of industrial dyes

- Dyes are widely used in different industries such as food, pharmaceutical, textile, printing, leather and cosmetic.
- This kind of processes results in discharging highly colored effluents that affect the solubility of the gas in water bodies and the transparency of water.
- Thus, it is essential to remove dyes before discharging these effluents into natural water streams.
- Among microorganisms, fungi are the most efficient in breaking down synthetic dyes

Microorganism	Dye	Immobilizing matrix
Phanerochaete sordida	Basic Blue 22	Plastic disks
Funalia trogii	Acid Black 52	Sodium alginate beads
Pycnoporus cinnabarinus	Remazol Brilliant Blue R	Nylon sponge
Phanerochaete crysosporium	Poly R-478	Grape seeds, wheat straw, wood shavings
Unidentified basidiomycetous	Orange II	Alginate beads
Bjerkandera adusta	Reactive Black 5	Plastic net
Trametes hirsuta	Indigo Carmine, Phenol Red	Alginate beads
Pseudomonas aeruginosa, *Aeromonas eucrenophila* and *Clavibacter michiganensis*	Methylene blue	Cellulose acetate Nano fibrous web
Pseudomonas putida and *Bacillus licheniformis*	reactive dyes (RR195, RO 72, RY 17, RB 36)	Sodium alginate and polyacrylamide gel beads
Microbial consortium	Azo dye (RED RBN)	Phosphorylated polyvinyl alcohol (PVA) gel
Bacillus subtilis	Methylene blue	Calcium alginate bead
Bacteria (*Pseudomonas aeruginosa*) and Fungal cells (*Phanerochate chrysosporium*)	Reactive dye Procion Blue 2G	Sodium alginate and polyvinyl alcohol
Bacillus firmus	Polyazo dye	Tubular polymeric gel

Electronmicrographs of the immobilized *Candida tropicalis* in the ceramic carrier. (Magnification × 3000.)

Electron micrograph of the immobilized *Saccharomyces cerevisiae* on CLP-Fe. (Magnification × 1800.)

Summary

- *Immobilized cells systems were found to offer a multitude of advantages over suspended cells systems.*
- *For example, cell immobilization enhances the microbial cell stability, allows continuous process operation, simplifies solid–liquid separation and increases the resistance against environmental harsh conditions.*
- *Several studies have been conducted over the years to investigate the efficiency of immobilized cells for the biodegradation of different types of wastewater contaminants.*
- *Various microorganisms showed the ability to biologically degrade these contaminants.*

- *In attempting to improve cell immobilization technology, a new topic under study is the addition of* ***nanomaterials*** *such as iron oxide nanoparticles; it has been proven to have the capability to improve the efficiency of immobilized cells.*
- *Given these points, immobilized cells technology is a promising topic to be further researched with the aim to develop new effective and low-cost carriers, increase biomass concentration and prolong the life of immobilization carriers.*