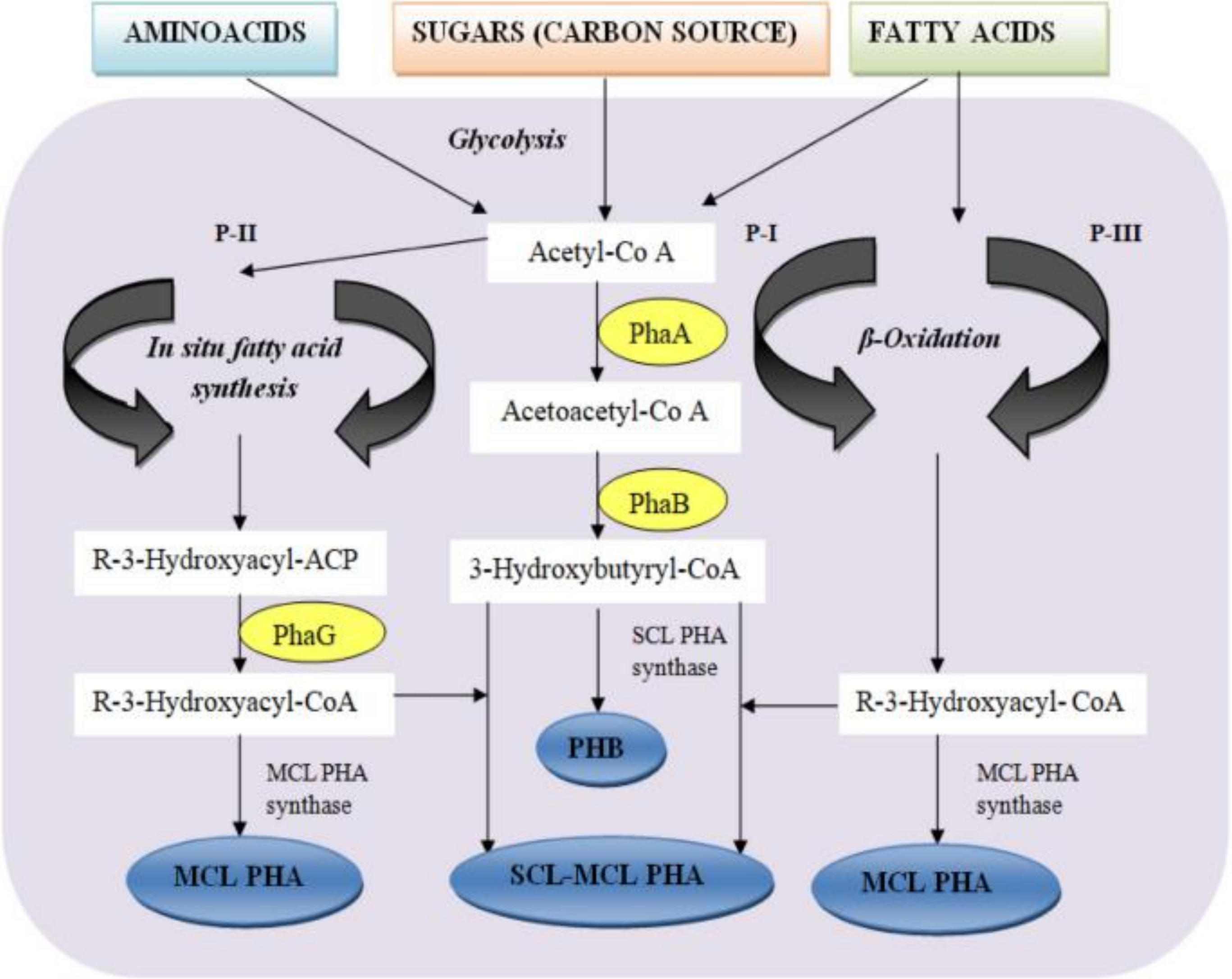
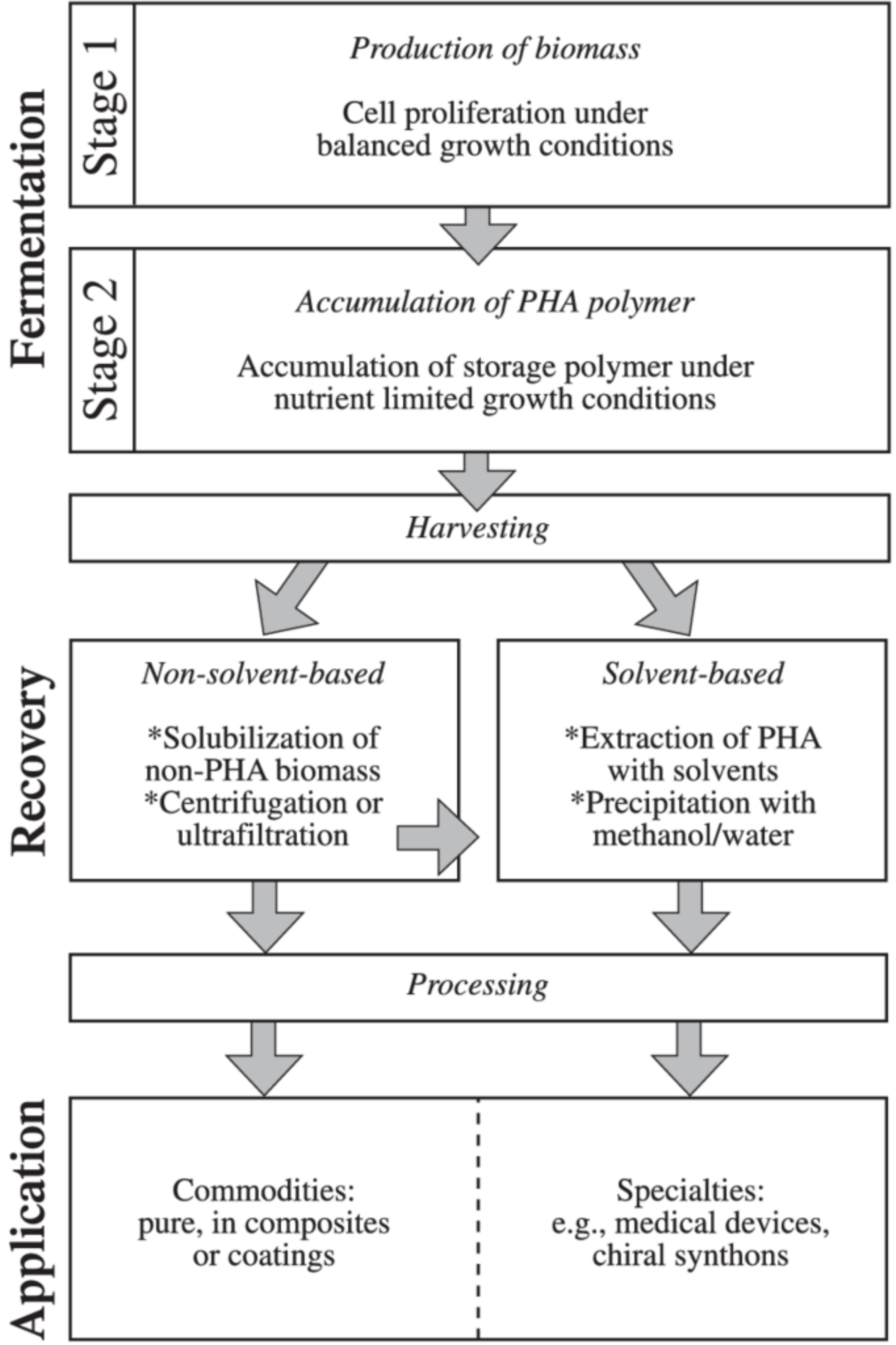
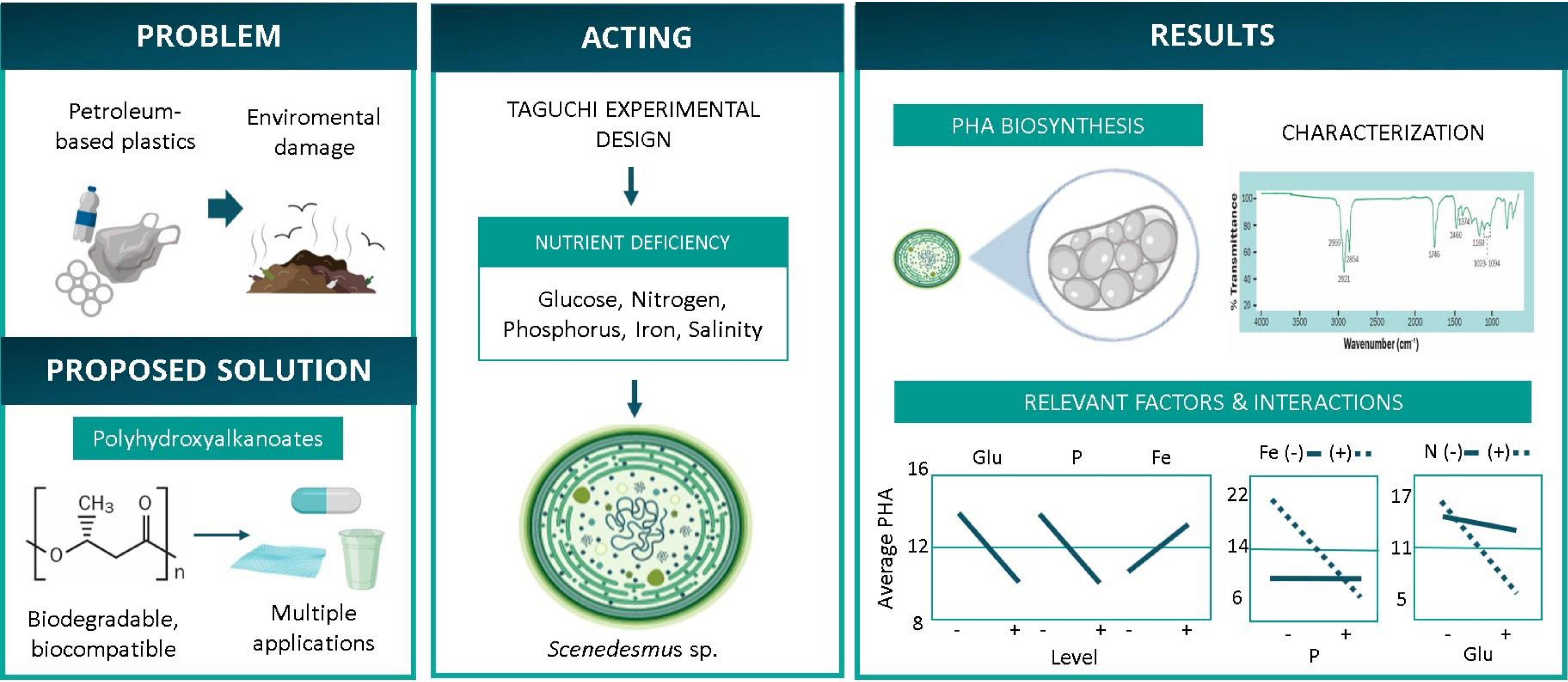
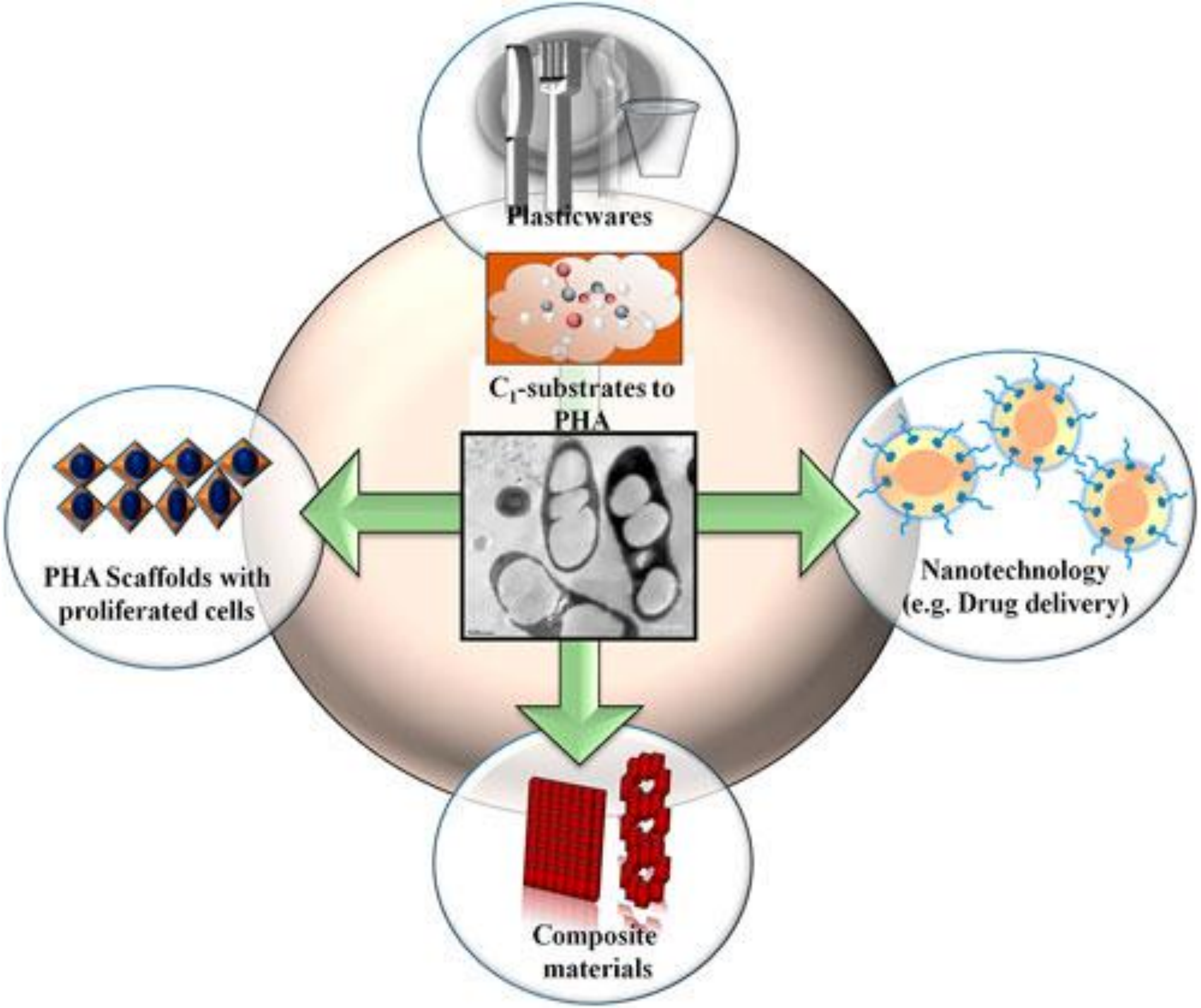


Biosynthetic Pathways of PHA

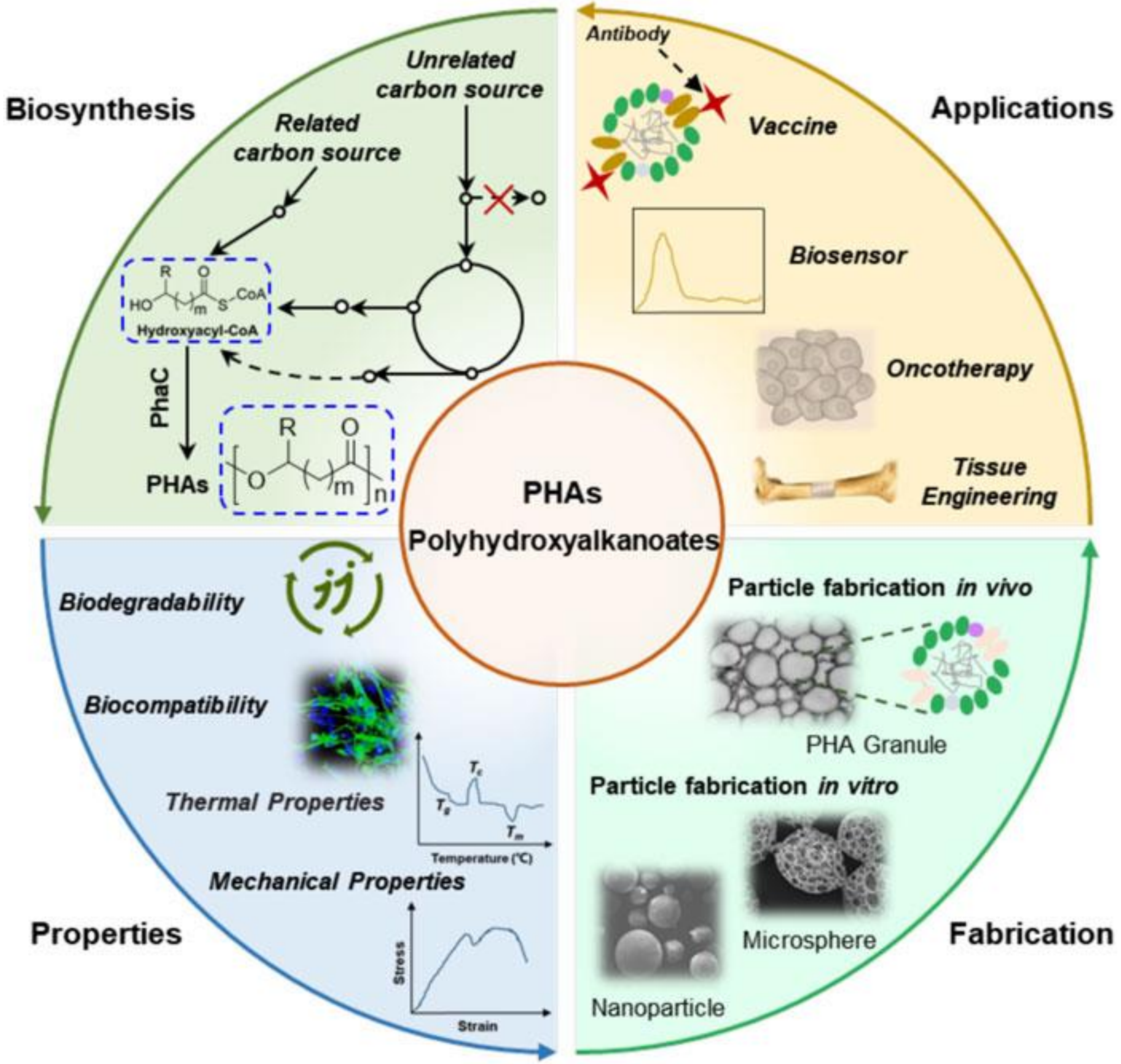
PHA production usually comprises three traditional pathways namely: Pathway I (P-I)—glycolysis, Pathway II (P-II)—in situ fatty acid synthesis, and Pathway III (P-III)—β-oxidation.


Fermentation
Stage 1
Production of biomass
Cell proliferation under balanced growth conditions
Stage 2
Accumulation of PHA polymer
Accumulation of storage polymer under nutrient limited growth conditions
Harvesting
Recovery
Non-solvent-based
*Solubilization of non-PHA biomass
*Centrifugation or ultrafiltration
Solvent-based
*Extraction of PHA with solvents
*Precipitation with methanol/water
Processing
Application
Commodities: pure, in composites or coatings
Specialties: e.g., medical devices, chiral synthons

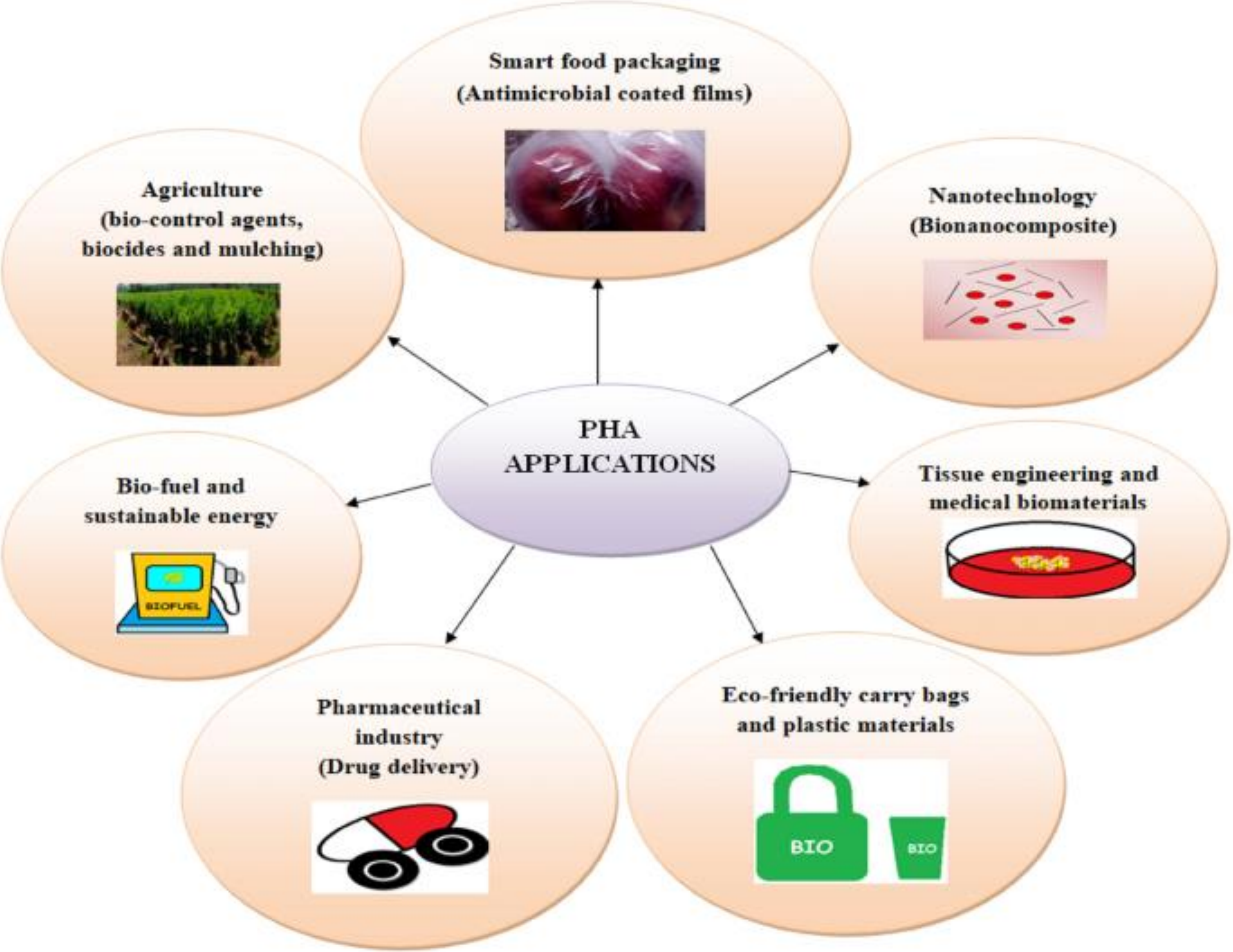

PROBLEM
Petroleum-based plastics
Enviromental damage
PROPOSED SOLUTION
Polyhydroxyalkanoates
CH3
O
O
n
Biodegradable, biocompatible
Multiple applications
ACTING
TAGUCHI EXPERIMENTAL DESIGN
NUTRIENT DEFICIENCY
Glucose, Nitrogen, Phosphorus, Iron, Salinity
Scenedesmus sp.
RESULTS
PHA BIOSYNTHESIS
CHARACTERIZATION
% Transmittance
Wavenumber (cm-1)
RELEVANT FACTORS & INTERACTIONS
Glu
P
Fe
Average PHA
Level
Fe (-) (+)
N (-) (+)
P
Glu



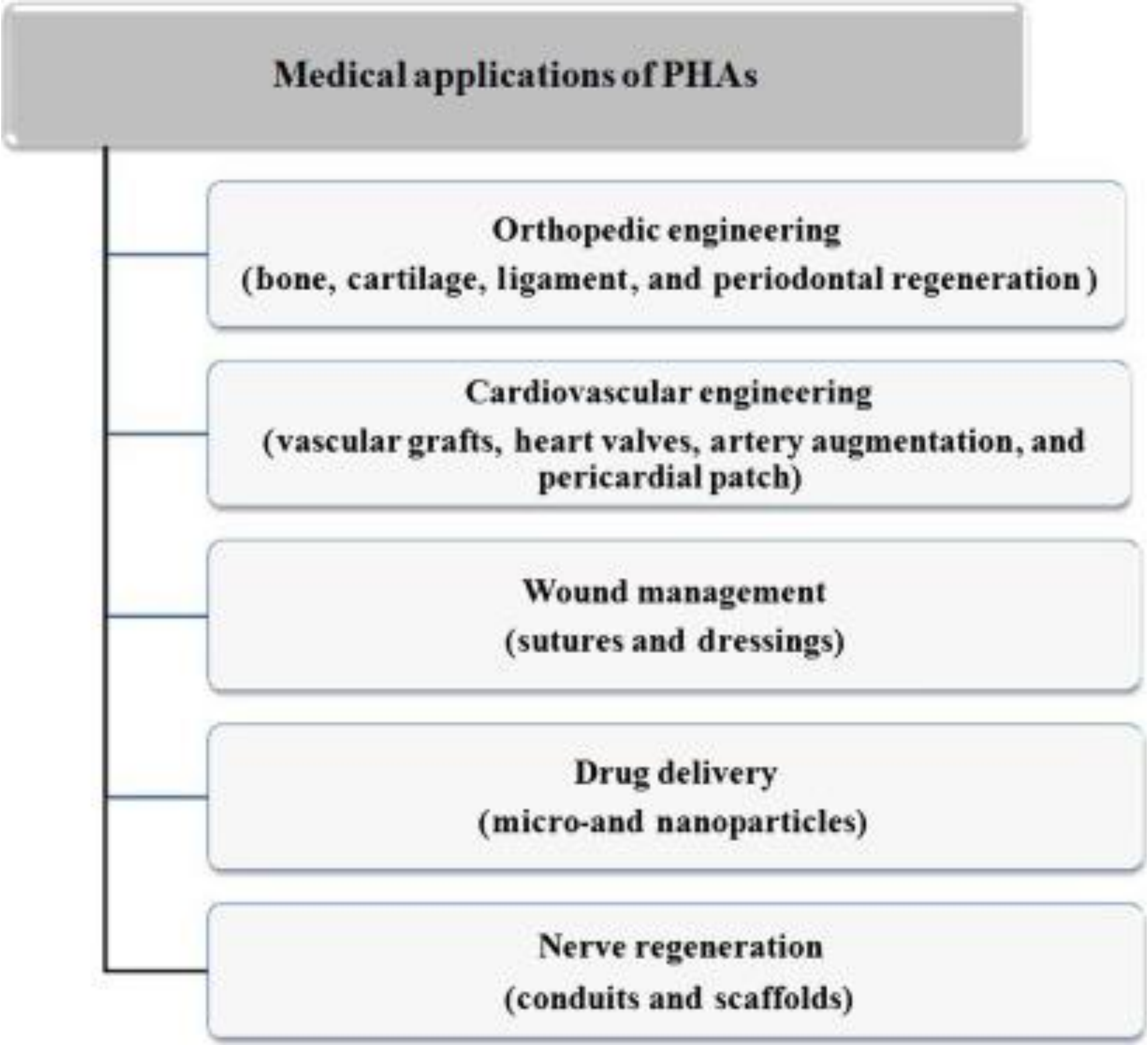
Plasticwares
C_1-substrates to PHA
PHA Scaffolds with proliferated cells
Nanotechnology (e.g. Drug delivery)
Composite materials



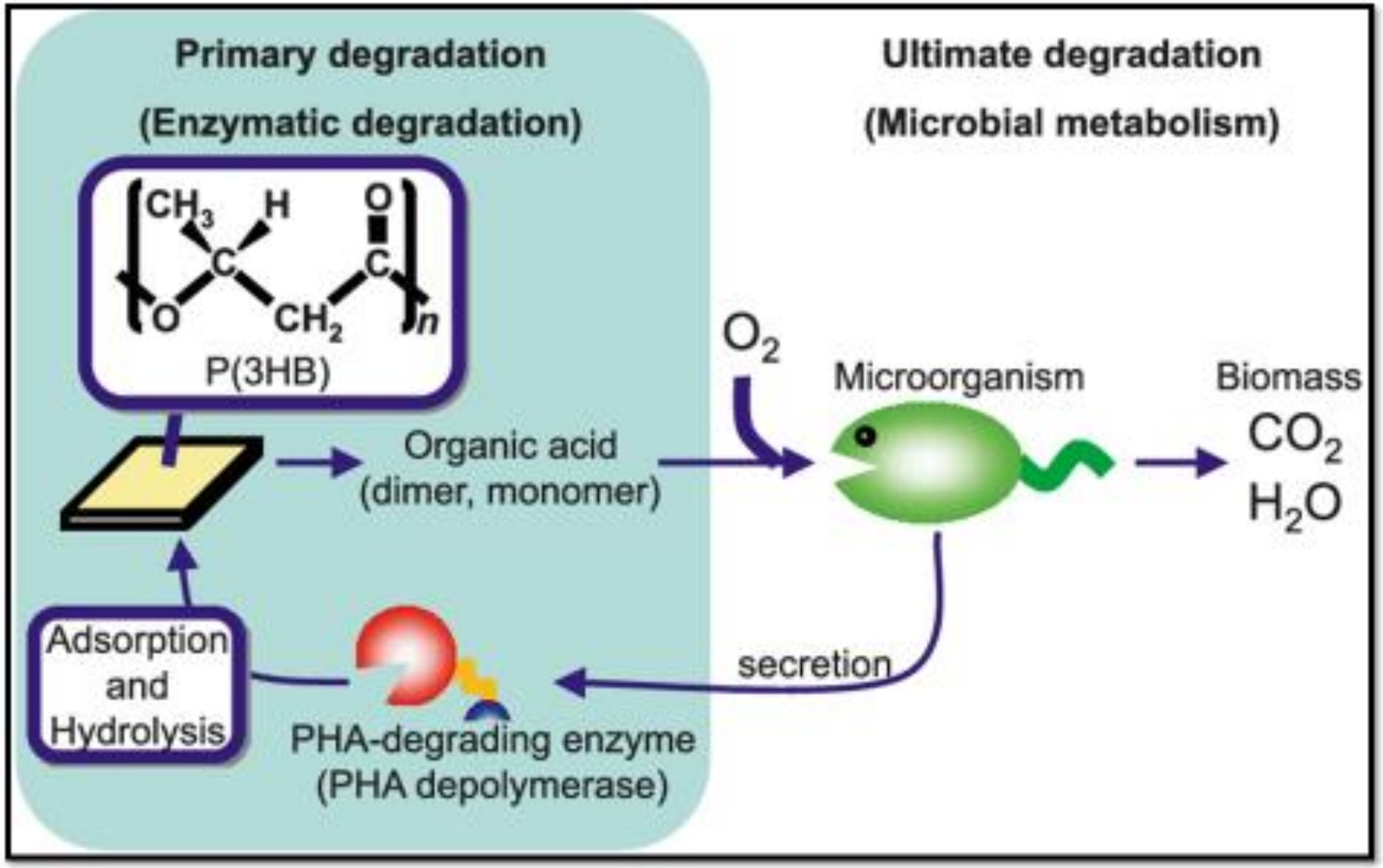
Biosynthesis
Unrelated carbon source
Related carbon source
Hydroxyacyl-CoA
PhaC
PHAs
Applications
Antibody
Vaccine
Biosensor
Oncotherapy
Tissue Engineering
PHAs
Polyhydroxyalkanoates
Biodegradability
Biocompatibility
Thermal Properties
Temperature (℃)
Mechanical Properties
Stress
Strain
Properties
Particle fabrication in vivo
PHA Granule
Particle fabrication in vitro
Microsphere
Nanoparticle
Fabrication



Smart food packaging
(Antimicrobial coated films)
Agriculture
(bio-control agents,
biocides and mulching)
Nanotechnology
(Bionanocomposite)
PHA
APPLICATIONS
Bio-fuel and
sustainable energy
BIOFUEL
Tissue engineering and
medical biomaterials
Pharmaceutical
industry
(Drug delivery)
Eco-friendly carry bags
and plastic materials
BIO
BIO


Medical applications of PHAs
Orthopedic engineering
(bone, cartilage, ligament, and periodontal regeneration)
Cardiovascular engineering
(vascular grafts, heart valves, artery augmentation, and pericardial patch)
Wound management
(sutures and dressings)
Drug delivery
(micro-and nanoparticles)
Nerve regeneration
(conduits and scaffolds)

Degradation of Polyhydroxyalkanoates (PHAs)



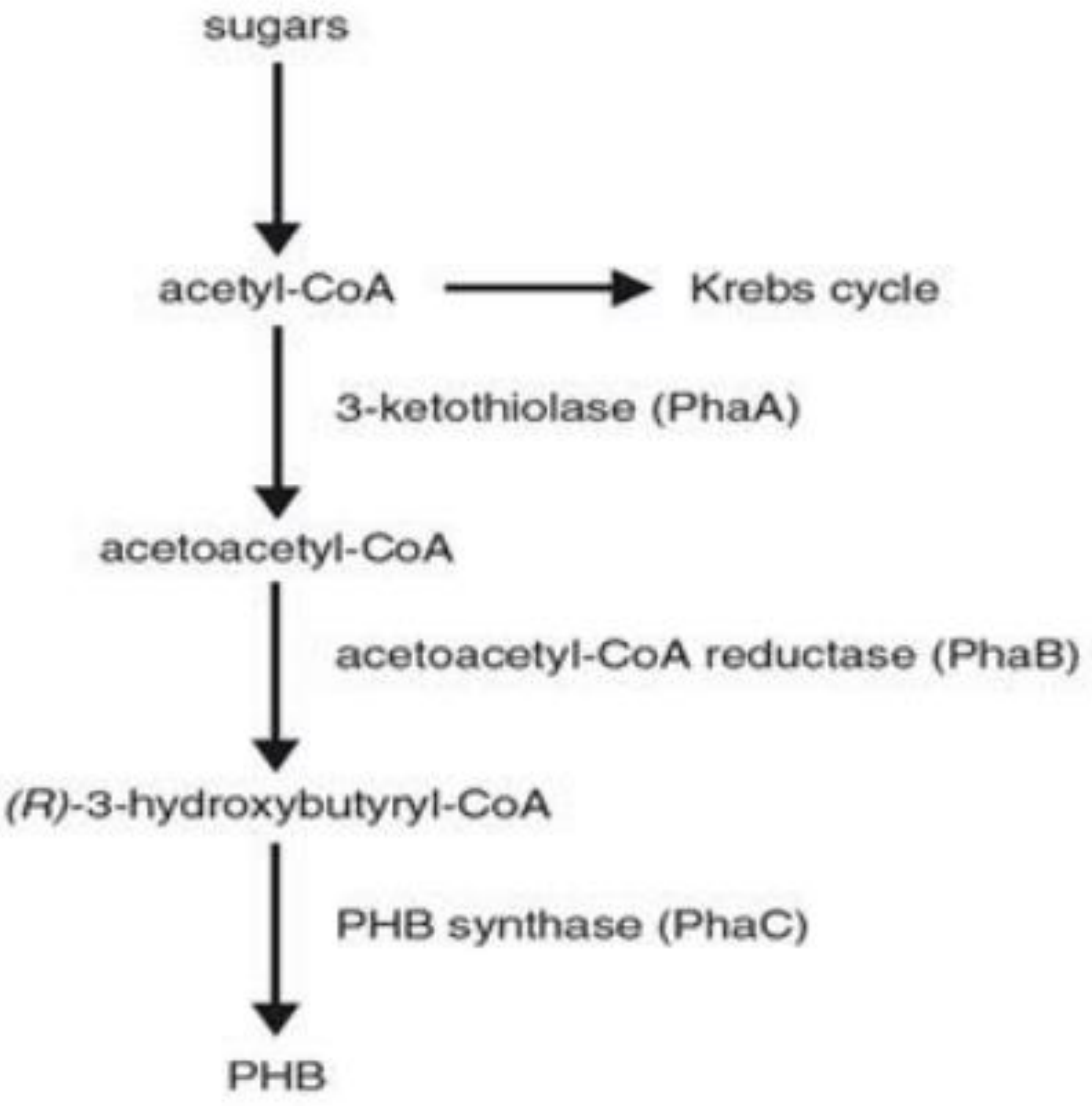
Among the most promising materials being developed and evaluated is polyhydroxybutyrate (PHB), a microbial bioprocessed polyester belonging to the polyhydroxyalkanoate (PHA) family.

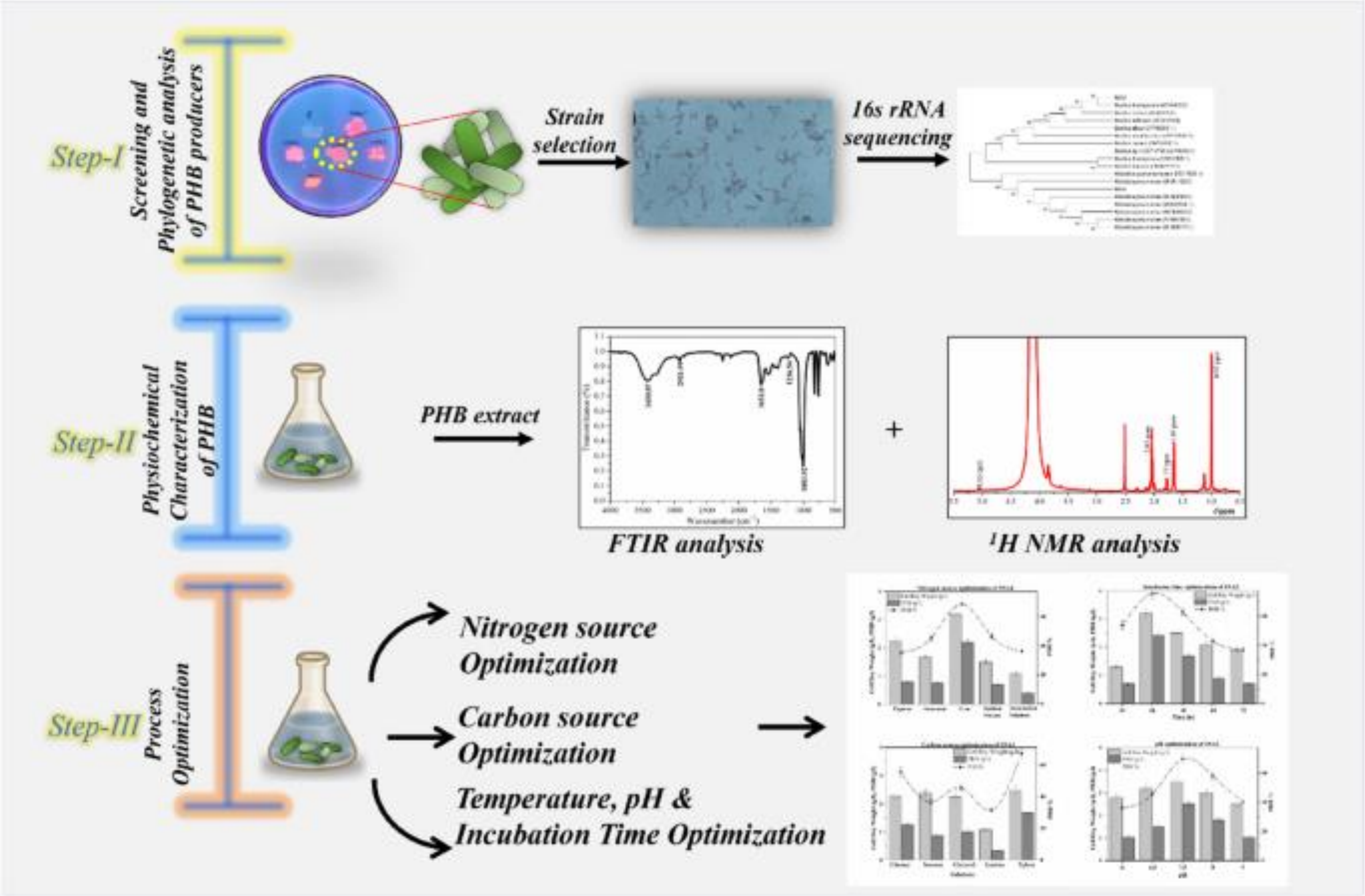
This biocompatible and non-toxic polymer is biosynthesized and accumulated by a number of specialized bacterial strains.

PHB is a stiff and relatively brittle polymer

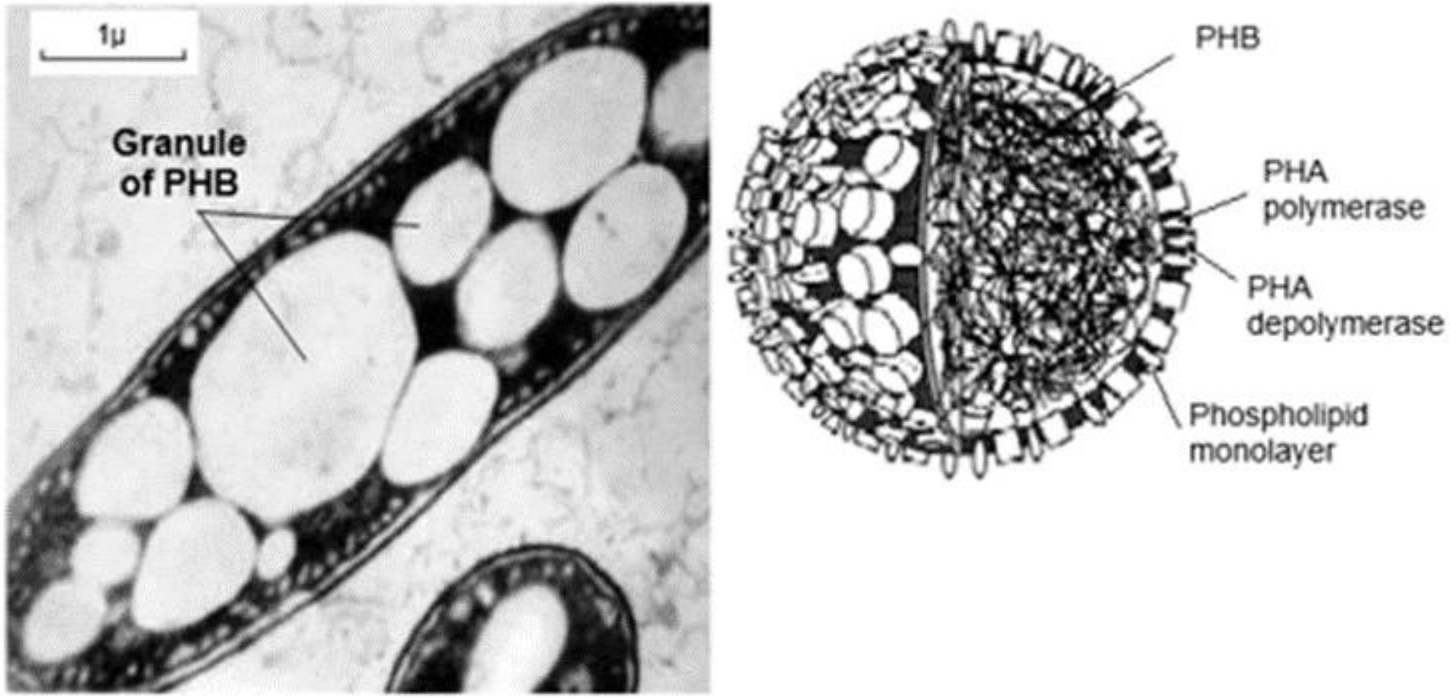
Polyhydroxylbutylene (PHB)

- PHB is produced by microorganisms such as *Ralstonia eutrophus*, *Bacillus megaterium*, in response to conditions of physiological stress mainly conditions in which nutrients are limited.
- The polymer is primarily a product of carbon assimilation (from glucose or starch) and is employed by micro-organism as a form of energy storage molecule to be metabolized when other common energy sources are not available.
- Microbial biosynthesis of PHB starts with the condensation of two molecules of acetyl CoA to give acetoacetyl-CoA which is subsequently reduced to hydrooxybutyryl-CoA.


sugars
acetyl-CoA
Krebs cycle
3-ketothiolase (PhaA)
acetoacetyl-CoA
acetoacetyl-CoA reductase (PhaB)
(R)-3-hydroxybutyryl-CoA
PHB synthase (PhaC)
PHB



Step-I
Screening and Phylogenetic analysis of PHB producers
Strain selection
16s rRNA sequencing
Step-II
Physiochemical Characterization of PHB
PHB extract
+
FTIR analysis
¹H NMR analysis
Step-III
Process Optimization
Nitrogen source Optimization
Carbon source Optimization
Temperature, pH & Incubation Time Optimization

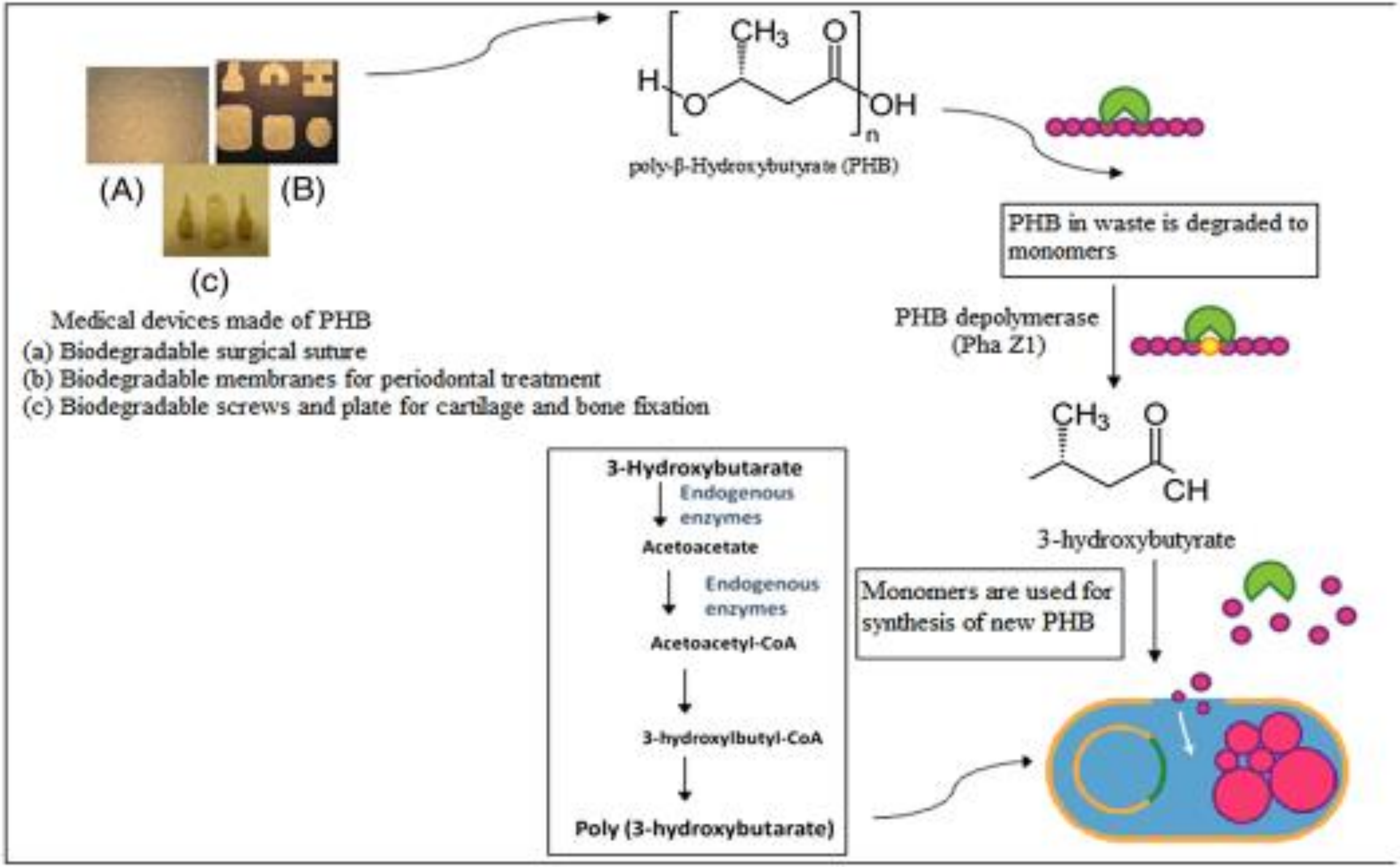

1µ
Granule
of PHB
PHB
PHA
polymerase
PHA
depolymerase
Phospholipid
monolayer

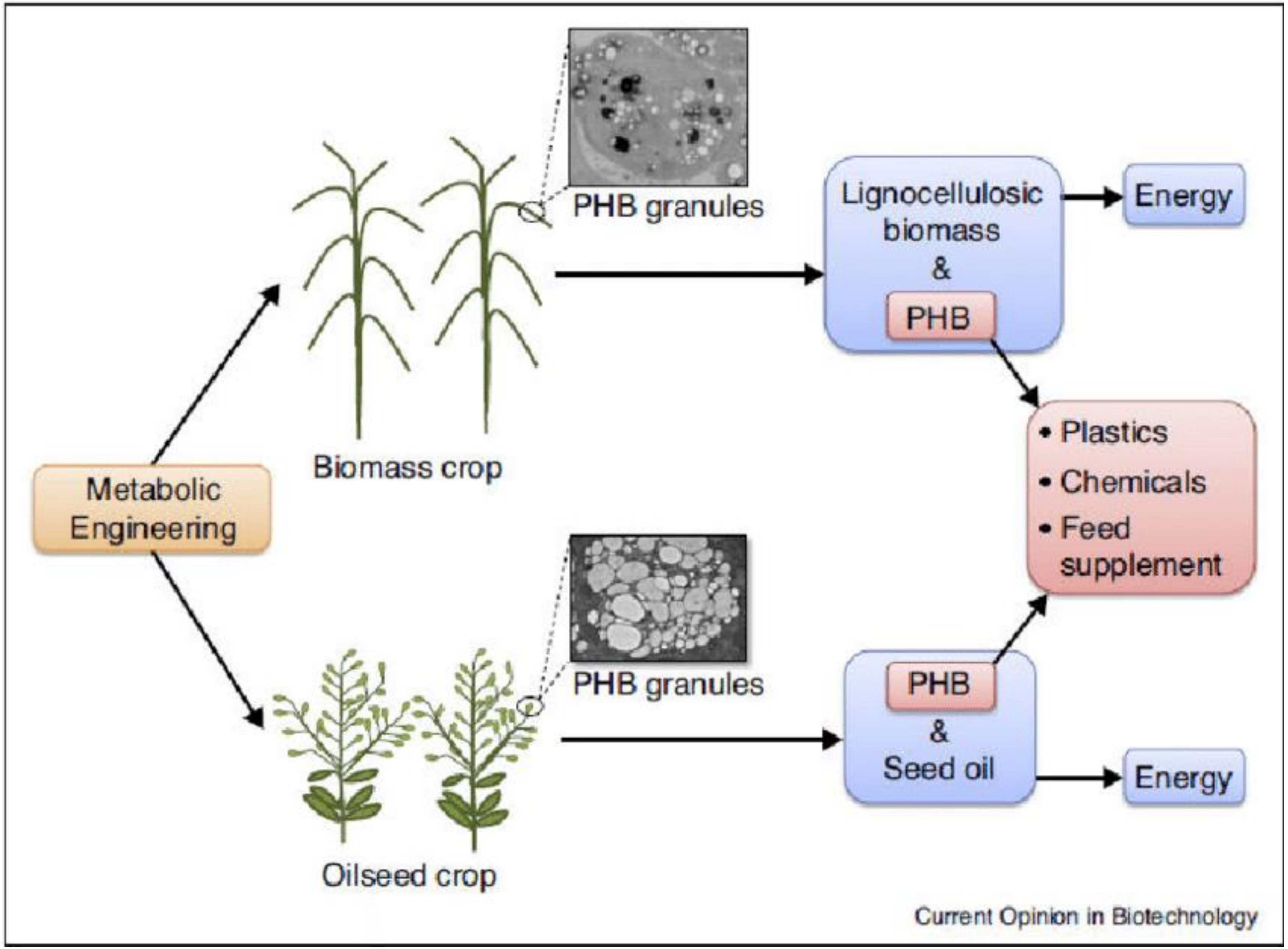
(a) (b)

Important PHB producing bacteria

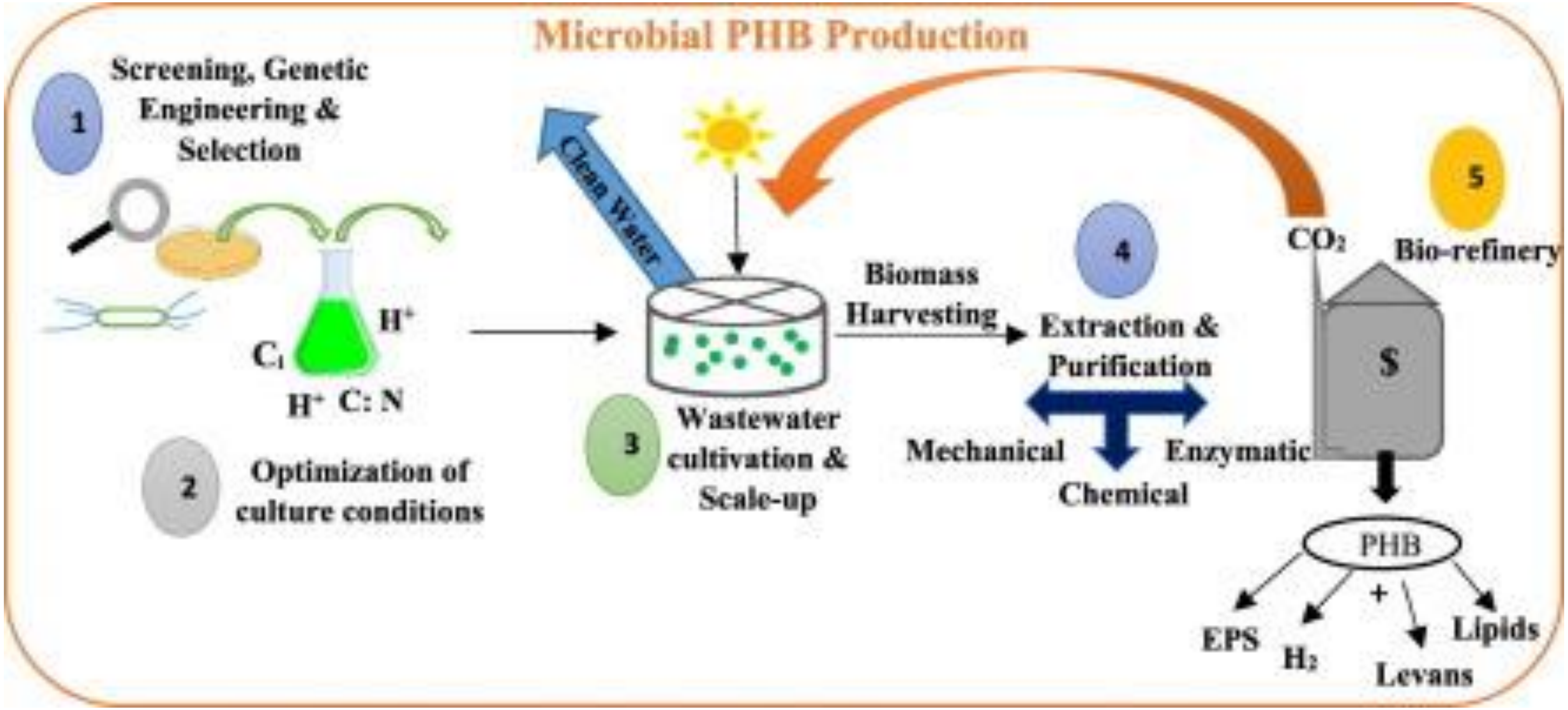
- *Ralstonia*
- *Bacillus*
- *Pseudomonas*
- *Alcaligenes*
- *Azotobacter*
- *Hydrogenomonas*
- *Chromatium*
- *Methylobacterium*
- Recombinant *Escherichia coli* and many others.

Synthesis of PHB

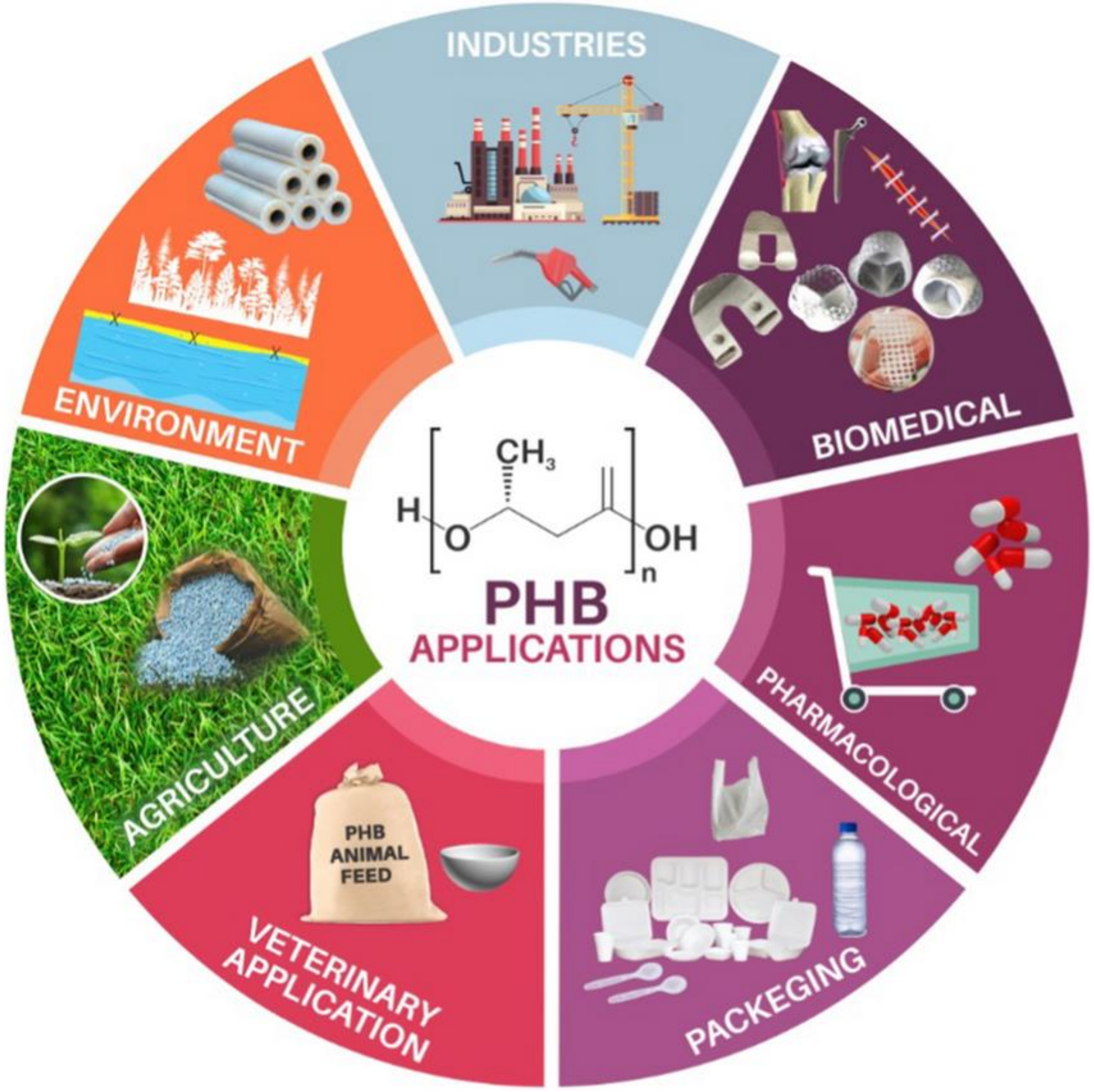




PHB granules
Lignocellulosic biomass & PHB
Energy
Biomass crop
Metabolic Engineering
Plastics
Chemicals
Feed supplement
PHB granules
PHB & Seed oil
Energy
Oilseed crop
Current Opinion in Biotechnology



Microbial PHB Production
1
Screening, Genetic Engineering & Selection
H+
C1
H+ C: N
2
Optimization of culture conditions
Clean Water
3
Wastewater cultivation & Scale-up
Biomass Harvesting
4
Extraction & Purification
Mechanical
Enzymatic
Chemical
CO2
5
Bio-refinery
$
PHB
+
EPS
H2
Levans
Lipids


INDUSTRIES
BIOMEDICAL
PHARMACOLOGICAL
PACKEGING
VETERINARY APPLICATION
PHB ANIMAL FEED
AGRICULTURE
ENVIRONMENT
PHB
APPLICATIONS
CH3
H
O
OH
n

Area	Application
Biomedical	Sutures, supports of tissue cultures for implants, surgical implants, dressings, part of bones and replanted veins, engineering of heart valves and pins.
Pharmacological	Encapsulation of medicines for controlled release
Packaging	Food packaging
Veterinary	Encapsulation of veterinary medicinal products
Agricultural	Encapsulation of fertilizers
Environmental	Bags, bottles, disposable items, items of personal hygiene, films of involvement, degradable diapers and remediation of areas affected by oil spills.
Industrial	Recovery of oligomers and monomers for new use in the synthesis of polymers

Other biodegradable polymers

- **Water soluble polymers**: of two types
  1. Polyvinyl alcohol (PVOH)
  2. Ethylene vinyl alcohol (EVOH)
- **Photo-biodegradable plastics:** thermoplastics synthetic polymers that degrade in the presence of UV radiation. They become weak and brittle when exposed to sunlight for prolonged periods.

Microorganisms used for degrading some polymers

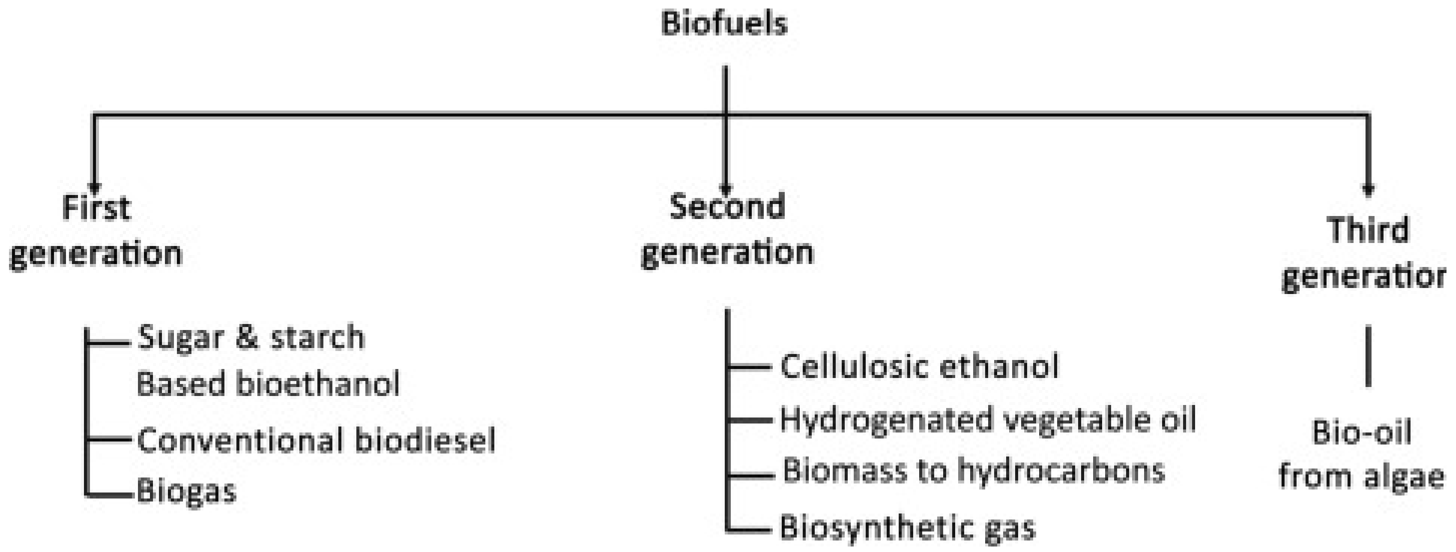
- PHA (polyhydroxy alkanoates) degraded by ***Streptomyces*** sp.
- PLA (polylactic acid) degraded by ***Amycolatopsos*** sp. And ***Saccharotrix*** sp.
- PCL (polycaprolactone) and PBS (polybutylene succinate) degraded by bacterial strains of genus ***Clostridium*** under aerobic conditions.
- PHB (polyhydroxylbutylene) degraded by ***Bacillus, Pseudomonas*** and ***Streptomyces*** sp.
- AAC (aliphatic-aromatic copolymers) degraded by ***Xanthomonas*** sp.

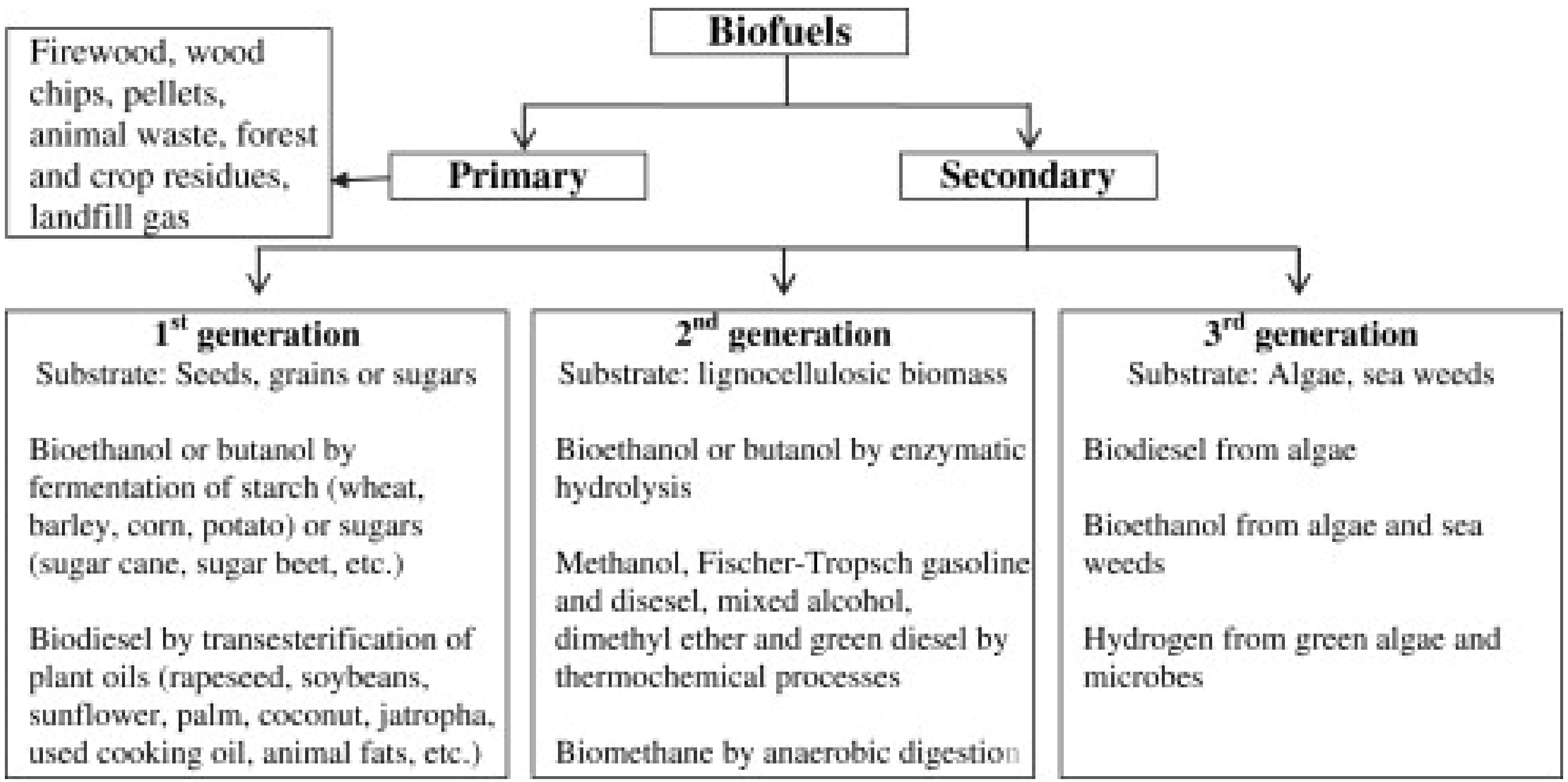
Biofuel

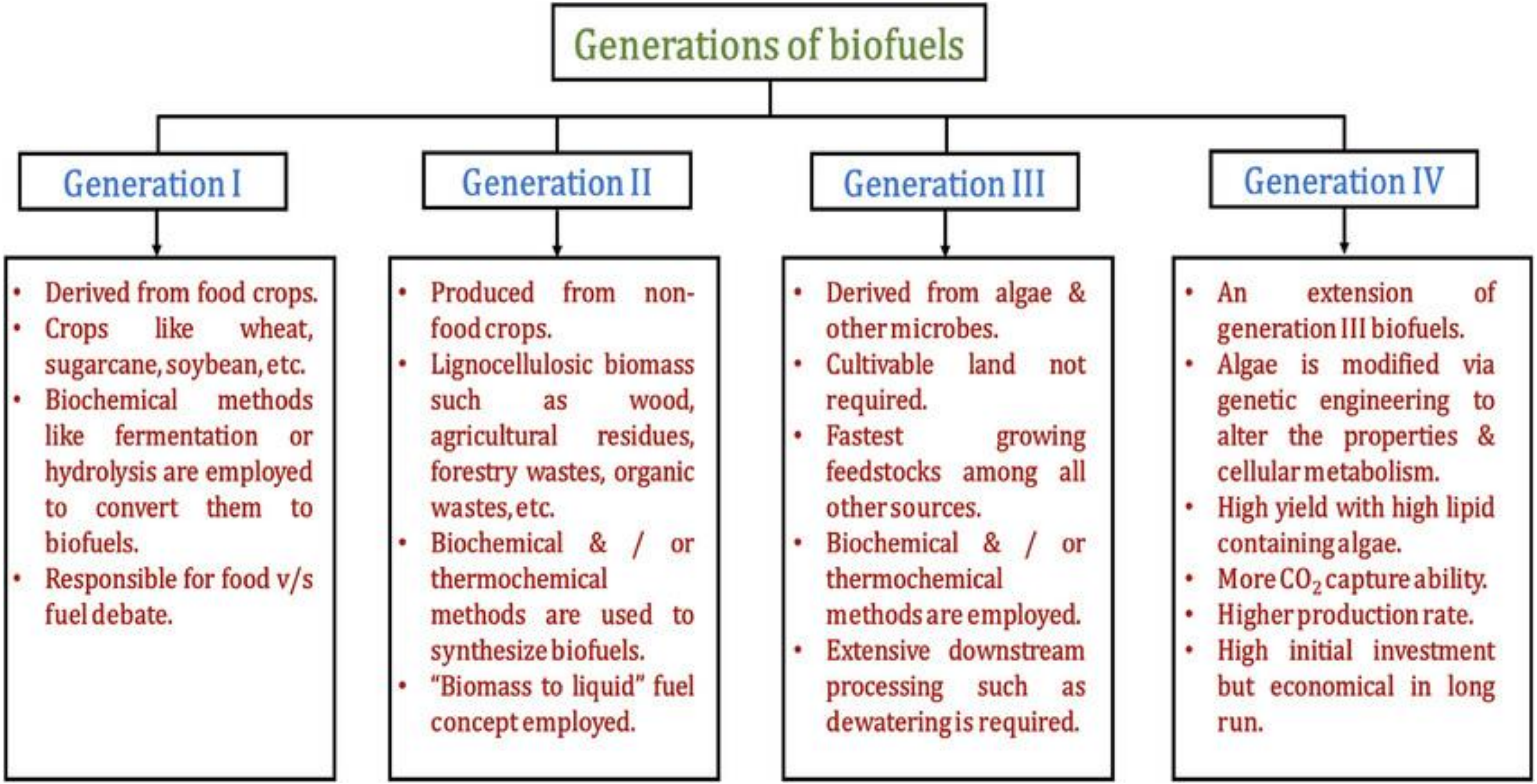
- **A biofuel is a fuel that is produced through contemporary processes from biomass**, rather than a fuel produced by the very slow geological processes involved in the formation of fossil fuels, such as oil. Since biomass technically can be used as a fuel directly (e.g. wood logs), some people use the terms biomass and biofuel interchangeably.
- The two most common types of biofuel are bioethanol and biodiesel.
  - **Bioethanol** is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn, sugarcane, or sweet sorghum. Cellulosic biomass, derived from non-food sources, such as trees and grasses, is also being developed as a feedstock for ethanol production. Ethanol can be used as a fuel for vehicles in its pure form (E100), but it is usually used as a gasoline additive to increase octane and improve vehicle emissions. Bioethanol is widely used in the United States and in Brazil.
  - **Biodiesel** is produced from oils or fats using transesterification and is the most common biofuel in Europe. It can be used as a fuel for vehicles in its pure form (B100), but it is usually used as a diesel additive to reduce levels of particulates, carbon monoxide, and hydrocarbons from diesel-powered vehicles.

- Examples of biofuels include ethanol (often made from corn in the United States and sugarcane in Brazil), biodiesel (sourced from vegetable oils and liquid animal fats), green diesel (derived from algae and other plant sources), and biogas (methane derived from animal manure and other digested organic material).

- There are three types of biofuels: 1st, 2nd and 3rd generation biofuels. They are characterized by their sources of biomass, their limitations as a renewable source of energy, and their technological progress. The main drawback of 1st generation biofuels is that they come from biomass that is also a food source.

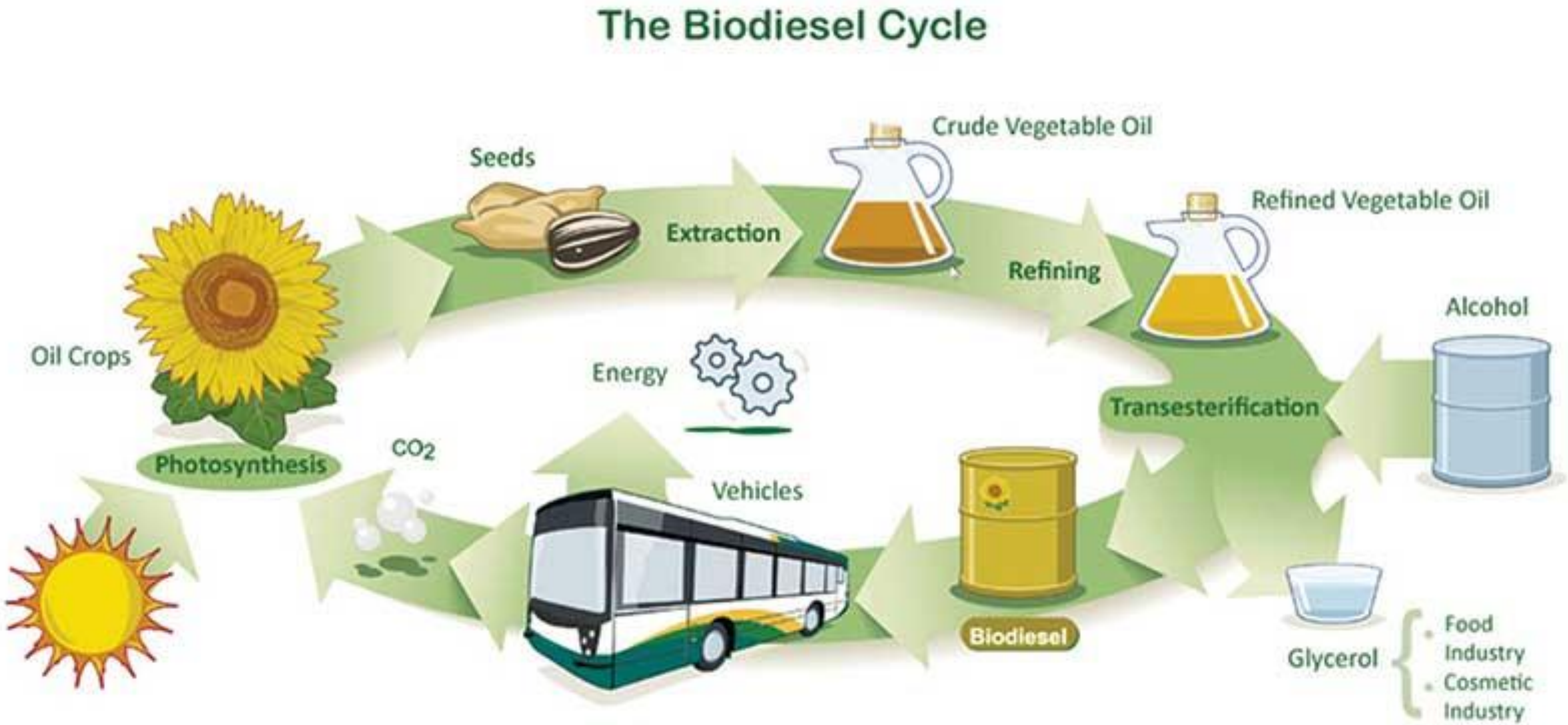

Biofuels
First generation
Second generation
Third generatior
Sugar & starch Based bioethanol
Conventional biodiesel
Biogas
Cellulosic ethanol
Hydrogenated vegetable oil
Biomass to hydrocarbons
Biosynthetic gas
Bio-oil from algae

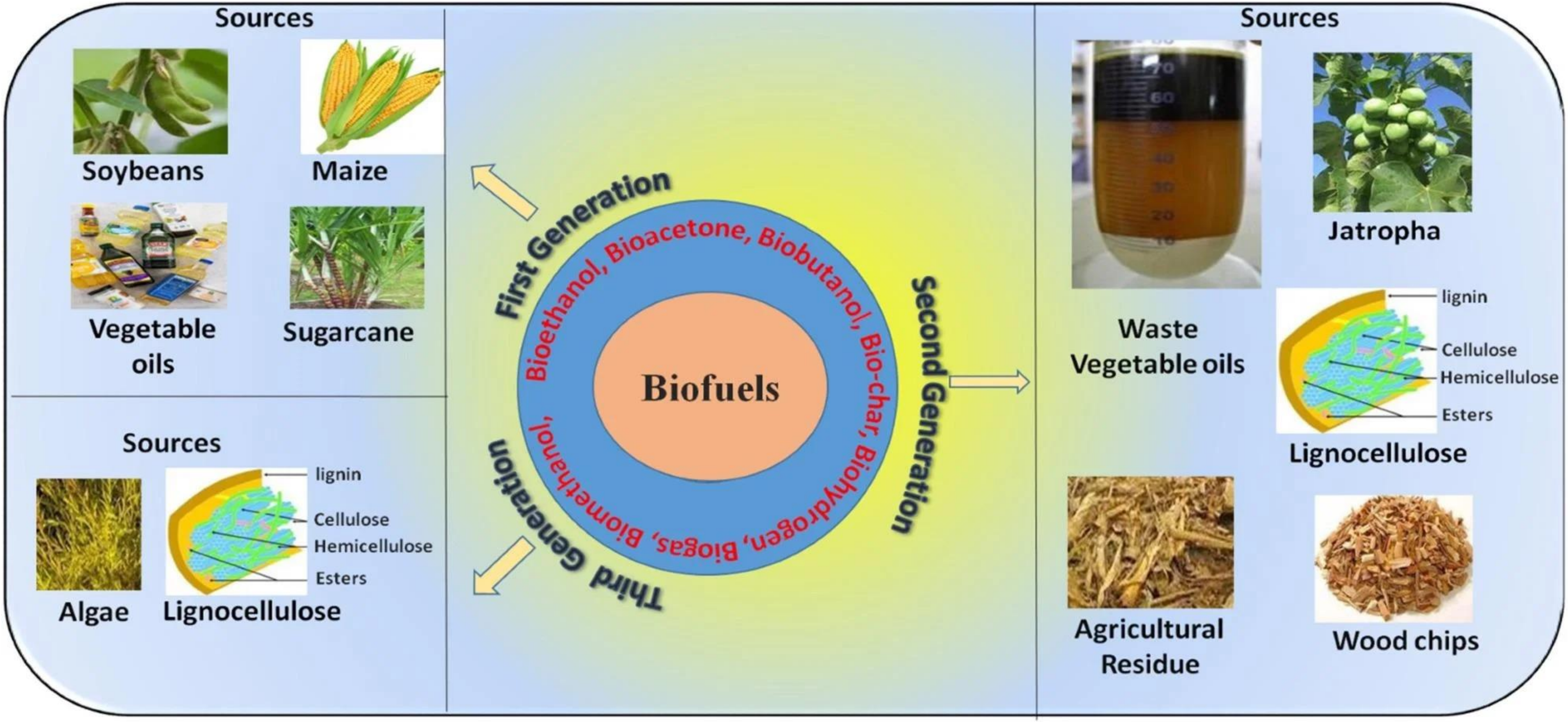

Biofuels
Primary
Secondary
Firewood, wood chips, pellets, animal waste, forest and crop residues, landfill gas
1st generation
Substrate: Seeds, grains or sugars
Bioethanol or butanol by fermentation of starch (wheat, barley, corn, potato) or sugars (sugar cane, sugar beet, etc.)
Biodiesel by transesterification of plant oils (rapeseed, soybeans, sunflower, palm, coconut, jatropha, used cooking oil, animal fats, etc.)
2nd generation
Substrate: lignocellulosic biomass
Bioethanol or butanol by enzymatic hydrolysis
Methanol, Fischer-Tropsch gasoline and diesel, mixed alcohol, dimethyl ether and green diesel by thermochemical processes
Biomethane by anaerobic digestion
3rd generation
Substrate: Algae, sea weeds
Biodiesel from algae
Bioethanol from algae and sea weeds
Hydrogen from green algae and microbes



Generations of biofuels
Generation I
• Derived from food crops.
• Crops like wheat, sugarcane, soybean, etc.
• Biochemical methods like fermentation or hydrolysis are employed to convert them to biofuels.
• Responsible for food v/s fuel debate.
Generation II
• Produced from non-food crops.
• Lignocellulosic biomass such as wood, agricultural residues, forestry wastes, organic wastes, etc.
• Biochemical & / or thermochemical methods are used to synthesize biofuels.
• "Biomass to liquid" fuel concept employed.
Generation III
• Derived from algae & other microbes.
• Cultivable land not required.
• Fastest growing feedstocks among all other sources.
• Biochemical & / or thermochemical methods are employed.
• Extensive downstream processing such as dewatering is required.
Generation IV
• An extension of generation III biofuels.
• Algae is modified via genetic engineering to alter the properties & cellular metabolism.
• High yield with high lipid containing algae.
• More CO_2 capture ability.
• Higher production rate.
• High initial investment but economical in long run.

Plants used as a biofuel production




Sources
Soybeans
Maize
Vegetable oils
Sugarcane
First Generation
Bioethanol, Bioacetone, Biobutanol, Bio-char, Biohydrogen, Biogas, Biomethanol,
Biofuels
Second Generation
Third Generation
Sources
Waste Vegetable oils
Jatropha
lignin
Cellulose
Hemicellulose
Esters
Lignocellulose
Agricultural Residue
Wood chips
Sources
Algae
lignin
Cellulose
Hemicellulose
Esters
Lignocellulose