BHARATHIDASAN UNIVERSITY

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Programme: M.Sc. Microbiology

COURSE TITLE: EXTREMOPHILES

Course Code: 24MICCBE2E

Unit – II: THERMOPHILES

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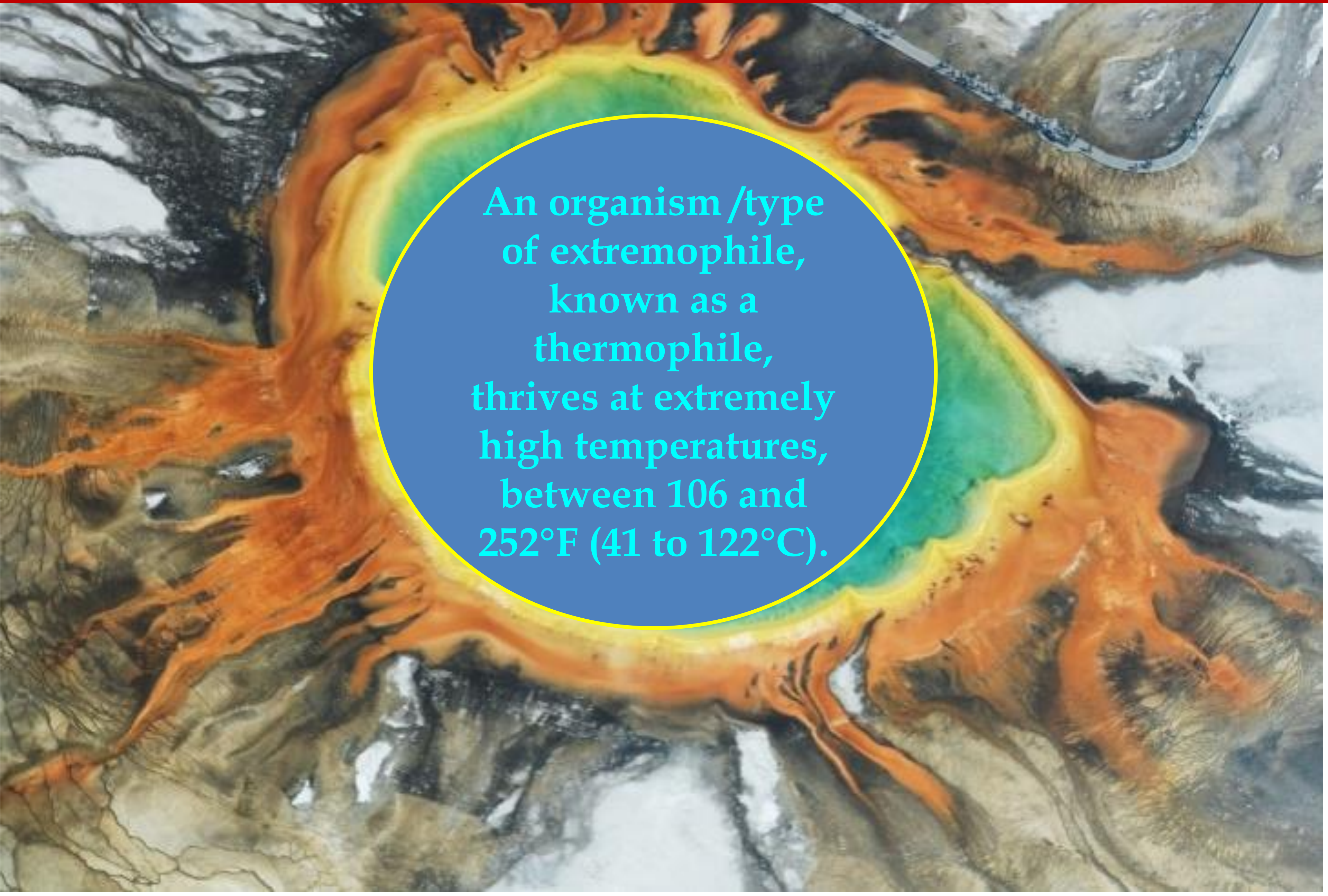
THERMOPHILES



An organism /type of extremophile, known as a thermophile, thrives at extremely high temperatures, between 106 and 252°F (41 to 122°C).

- Hyperthermophiles can grow even at 113°C-122°C
- Many thermophiles are archaea, though some of them are bacteria & fungi.
- Thermophilic eubacteria are suggested as an earliest bacteria of the earth.
- Thermophiles are found in various geothermally heated regions of the earth, such as hot springs like those in **Yellowstone National Park** and **deep sea hydrothermal vents,** as well as **decaying plant matter,** such as **peat bogs** (dense wetlands filled with partially decayed vegetation) & **compost.**

Classification

Thermophiles can be classified in various ways. One classification sorts these organisms according to their optimal growth temperatures:

Simple thermophiles: 50–64 °C (122–147 °F)

Extreme thermophiles 65–79 °C (149–174 °F)

Hyperthermophiles 80 °C (176 °F) & beyond, but not below 50 °C (122 °F)

In a related classification, thermophiles are sorted as follows:

1. **Facultative thermophiles (also called moderate thermophiles) can thrive at high temperatures, but also at lower temperatures [below 50°C (122 °F)], whereas**
2. **Obligate thermophiles (also called extreme thermophiles) require such high temperatures for growth.**
3. **Hyperthermophiles are particularly extreme thermophiles for which the optimal temperatures are above 80°C (176 °F).**

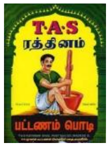
Geothermal areas are extremely varied in terms of geology and chemistry, but belong mainly to two categories:

first, the **solfatara** type (natural volcanic steam vent), characterized by fields with much S, acidic soils, acidic hot springs, and boiling mud pots;

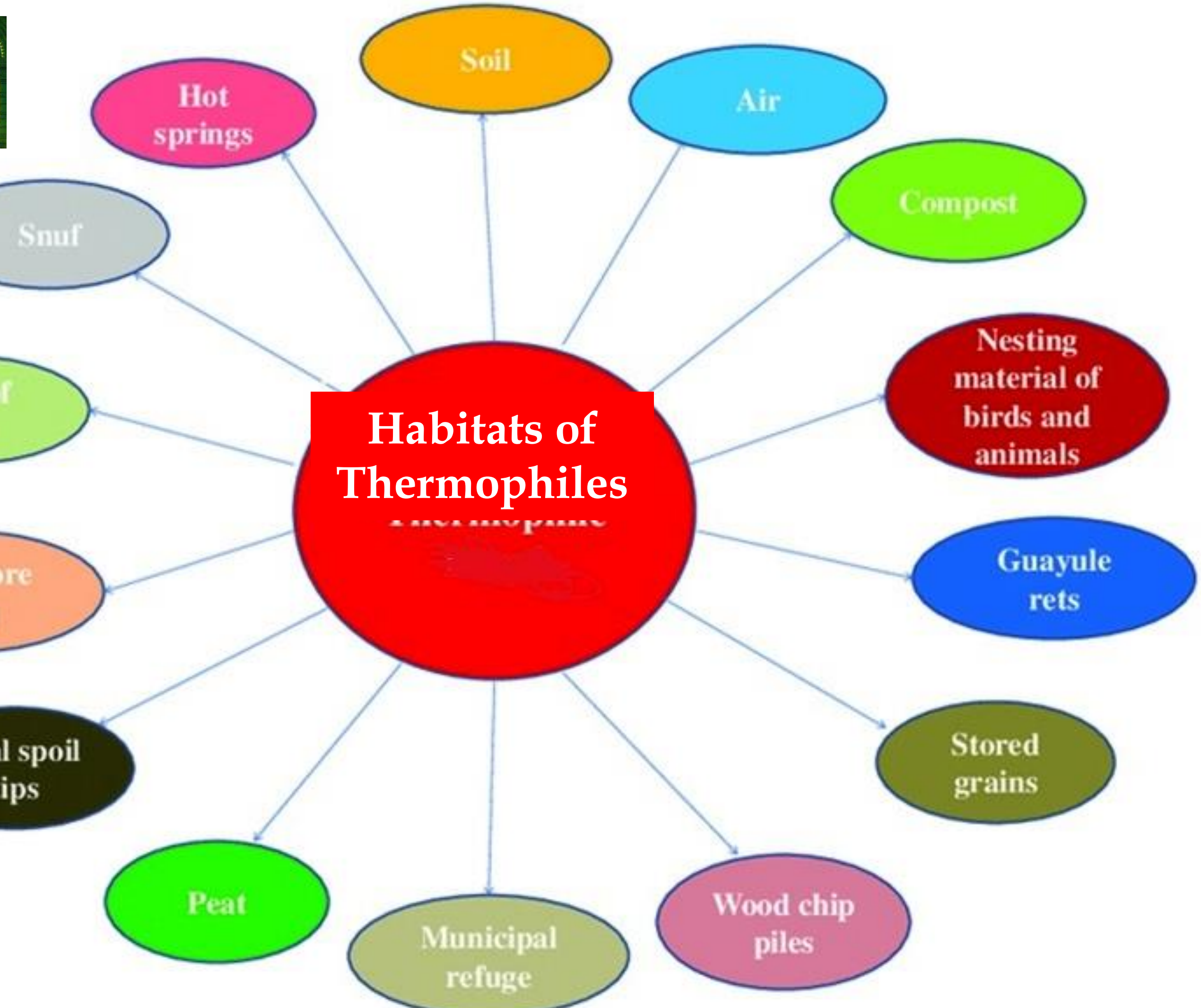
second, the **neutral-alkaline** type, characterized by freshwater hot springs and geysers, which are neutral to alkaline in pH.

Habitats of Thermophiles

A type of smokeless tobacco – made finely ground/ shredded leaves



Farm haystacks



Parthenium argentatum, commonly known as the ***guayule***

Common environmental niches that harbor thermophilic microbial consortia

Extremely Thermophilic Archaebacteria

- ***Thermoplasma*** **is an archeon without cell wall and live in hot acid coal refuse piles.**
- **First and the most hyperthermophilic organism isolated by Brock in 1977 is *Pyrolobus fumarii (Crenarchaeota)* from archaea, which has the optimum growth temperature at 106°C & at 113°C.**
- **The archaea (*Pyrococcus, Thermococcus, Sulpholobus etc.)* can have temperature optimum up to 142°C (Rothschild & Mancinelli, 2001).**

Extremely Thermophilic Archaebacteria

- **Two species of anaerobic heterotrophic archaea, (*Picrophilus oshimae & P. torridus*) were isolated from Japanese soils penetrated with solfataric gases, and show an optimal growth at pH 0.7 & 60°C.**
- **Sulphur metabolizers;** *Archaeoglobus profundus* a sulphur reducer bears optimum growth **temperature of 82°C.**

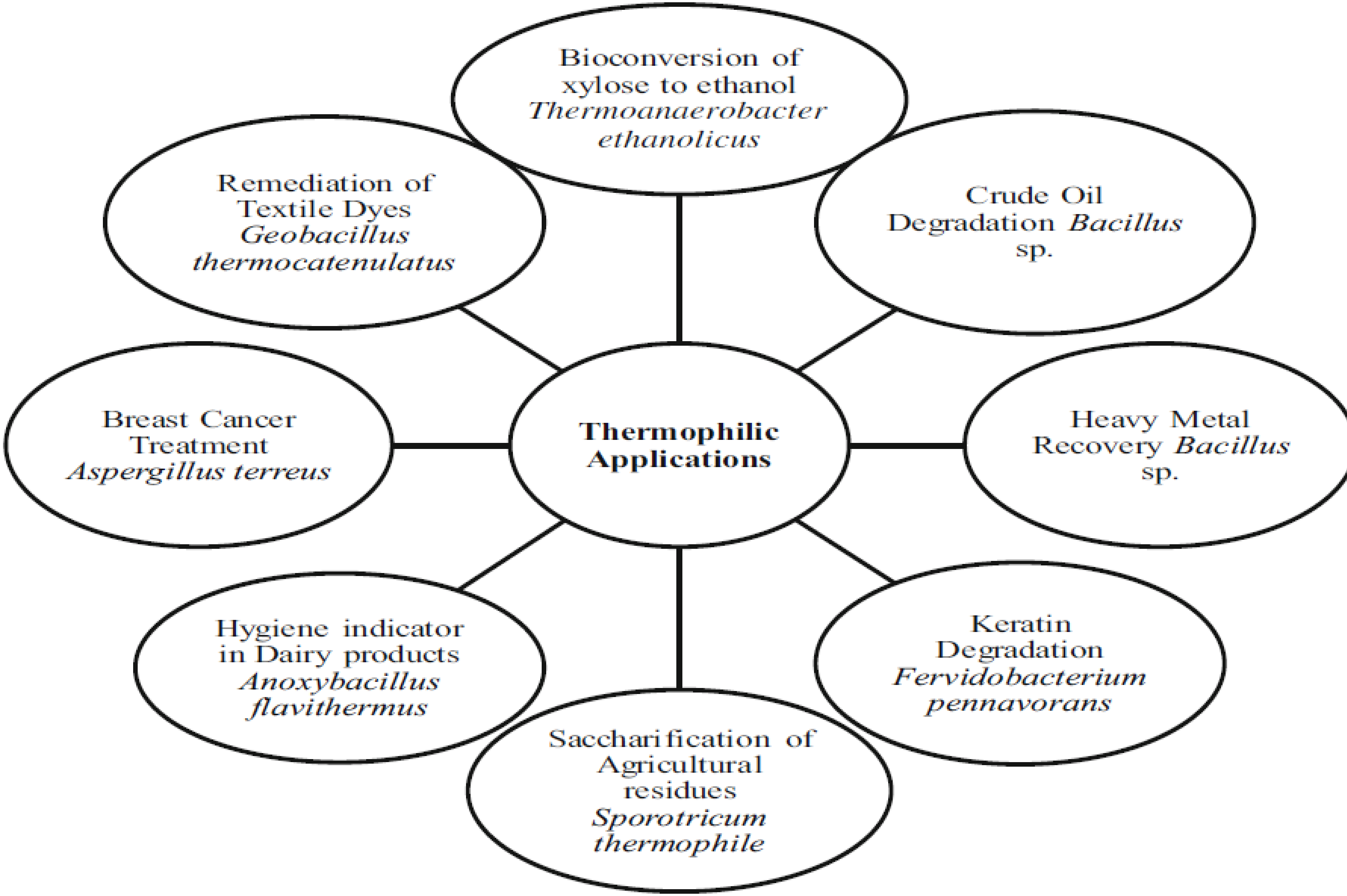
Thermophily

- A phenomenon, established by Thomas Brock through his extensive and pioneering studies in Yellowstone National Park (Wyoming, USA) from 1968 to 1978.
- **the ability/** phenomenon **of an organism to grow at a high temperature**.
- The condition of being a thermophile…..

Organism group	Maximum growth temperature °C
Animals	
Fish	38
Insects	45–50
Crustacea	48–50
Plants	
Vascular plants	45
Bryophytes	50
Eukaryotic microorganisms	
Protozoa	56
Algae	55–60
Fungi	60–62
Prokaryotes	
Cynaobacteria	70–72
Green bacteria	70–72
Bacteria	95
Archaea	113

Maximum growth temperature for main groups of organisms

Various applications of thermophilic microorganisms



Bioconversion of lignocellulose to H: *Clostridium thermocellum; Cl. thermocellum; s*everal species of genus *Thermoanaerobacterium*

Conversion of glycerol to lactate: Enzymes derived from thermophiles and hyperthermophiles.

Conversion of D-xylose into ethanol: *Thermoanaerobacter ethanolicus, Clostridium thermocellum; Cl. thermohydrosulfuricum* (reclassified as *Thermoanaerobacter thermohydrosulfuricus), Thermoanaerobium brockii* (reclassified as *Thermoanaerobacter brockii), Clostridium thermosaccharolyticum* (reclassified as *hermoanaerobacteriumthermosaccharolyticum*) and *Thermoanaerobacterium saccharolyticum* B6A.

Biodegradation of petroleum hydrocarbons: *Bacillus*

Recovery of heavy metals: *Bacillus* family

Remediation of textile dyes: *Geobacillus thermocatenulatus* MS5

Saccharification of agricultural residues: *Sporotricum thermophile* LAR5

Dairy processing: *Anoxybacillus flavithermus* and *Geobacillus* spp., many strains of genera *Lactobacillus* and *Bifidobacterium,* as well as some enterococci & yeasts.

- **Keratin degradation & converted as amino acids: serine, cysteine and proline:** *Fervidobacterium pennavorans*.
- **Cancer treatment:** Asperjinone, a nor-neolignan & Terrein, a suppressor of ABCG2-expressing breast cancer cells were isolated from thermophile *Aspergillus terreus.*

Table 1 Thermophilic enzymes and their potential roles

Microorganisms	Enzymes	Temperature of activity	Applications	References
Pyrococcus woesei	alpha-Amylases	Topt. = 100 °C	Sugar industry and starch processing	Alqueres et al. (2007)
Thermococcus profundus DT5432	alpha-Amylases	Topt. = 80 °C	Sugar industry and starch processing	Eichler (2001), Antranikian et al. (2005)
Staphylothermus marines	Pullulanases	Topt. = 90–105 °C	Sugar industry and starch processing	Eichler (2001), Antranikian et al. (2005)
Thermoplasma acidophilum	Glucoamylases	Topt. = 90 °C	Sugar industry and starch processing	Eichler (2001), Antranikian et al. (2005)
Pyrococcus woesei	β-Galactosidases	Topt. = 93 °C	Production of milk with low lactose content	Dabrowski et al. (1998)
Pyrococcus furiosus Sulfolobales sp.	Cellulases	Topt. = 103 °C	Production of alcohol, fruit industry	Antranikian et al. (2005)
Pyrodictium abyssi	Xylanases	Topt. = 100–110 °C	Paper industry–bleaching of pulp	Egorova and Antranikian (2005), Eichler (2001)
Humicola lanuginosa strain Y-38	Lipases	Topt. = 65 °C	Laundry detergents	Arima et al. (1972)
Myceliophthora thermophila	Laccases	Topt. = 60 °C	Polymerization of phenolic compounds to humic substances	Chefetz et al. (1998)
Myceliophthora thermophila	Phytases	Topt. = 42–45 °C	Animal feed	Wyss et al. (1999)
Penicillium duponti	glucose-6-phosphate dehydrogenase	Topt. = 50 °C	Generation of NADPH for biosynthetic reactions	Broad and Shepherd (1970)
Bacillus lichniformis	Alcalase	Topt. = 60 °C	Component of protein-fortified soft drinks and dietetic food, helps in protein recovery from meat, fish and crustacean shell waste	Synowiecki (2008)

Commercial aspects of thermophiles

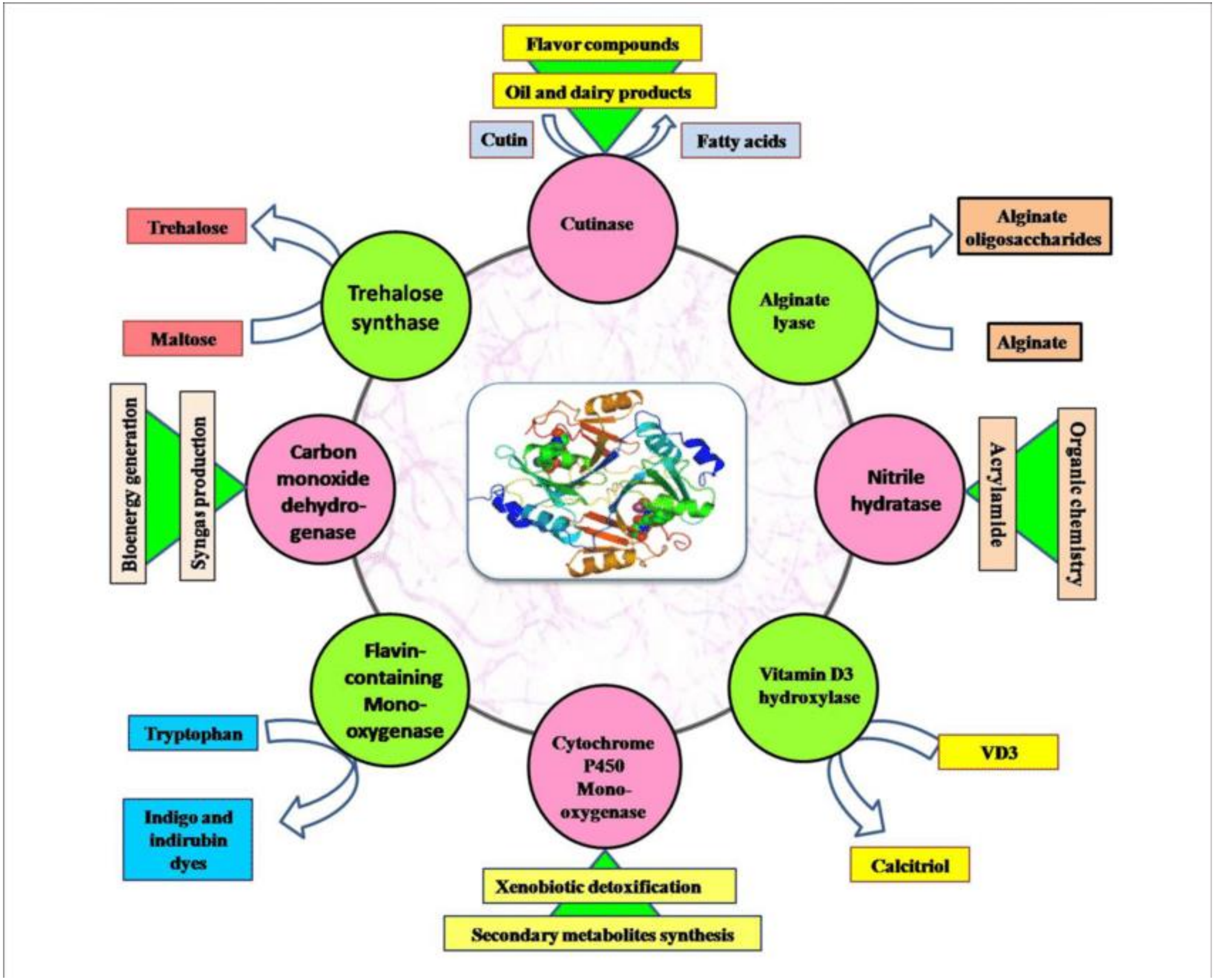


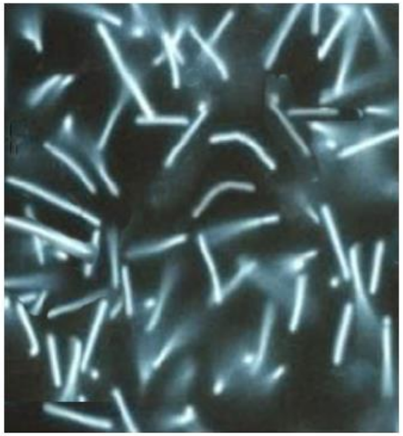
TABLE 9. Examples of thermophilic and hyperthermophilic enzymes with applications as molecular biology reagents

Enzyme	Origin	Applications	Properties	Source or reference(s)
Taq polymerase	*T. aquaticus*	PCR technologies	Optimal activity at 75°C, pH 9.0	26, 199
Vent DNA polymerase	*T. litoralis*		Optimal activity at 75°C, proofreading activity	2, 271
Deep Vent DNA polymerase	*P. furiosus*		Optimal activity at 75°C, proofreading activity; $t_{1/2}$, 23 h (95°C)	2, 343
C. therm DNA polymerase	*Carboxydothermus hydrogenoformans*	Reverse transcription-PCR	Reverse transcriptase activity, 3′→5′ proofreading activity; optimal activity as 60–70°C	Roche Molecular Biochemicals
DNA polymerase	*Thermus thermophilus*		Reverse transcriptase activity	Roche Molecular Biochemicals
Pfu DNA ligase	*P. furiosus*	Ligase chain reaction and DNA ligations	Active at 45–80°C; $t_{1/2}$ >60 min (95°C)	Stratagene
Tcs DNA ligase	*Thermus scodoductus*	Ligase chain reaction	Optimal activity at 45°C	Roche Molecular Biochemicals
DNA binding protein Ssod7	*S. solfataricus*	Time-reducing and specificity-enhancing in DNA-DNA hybridizations; locking of antisense oligonucleotide to target sequence	Sequence-aspecific DNA binding; ATP-independent, homology-dependent DNA annealing at 60°C	123
Serine protease (PreTaq)	*Thermus* strain Rt41A	DNA and RNA purifications; cellular structures degradation prior to PCR	Optimal activity at 90°C, pH 8.0; $t_{1/2}$, 20 min (90°C) (+$CaCl_2$)	Life Technologies
Protease S	*P. furiosus*	Protein fragmentation for sequencing	Optimal activity at 85–95°C, pH 6.0–8.0; 80% active after 3 h (95°C)	TaKaRa Biomedicals
Methionine aminopeptidase	*P. furiosus*	Cleavage of the N-terminal Met in proteins	Optimal activity at 85–95°C, pH 7.0–8.0; stable for 1 h (75°C)	TaKaRa Biomedicals
Pyroglutamate aminopeptidase	*P. furiosus*	Cleavage of the N-terminal L-pyroglutamate in proteins	Optimal activity at 95–100°C, pH 6.0–9.0; 95% active after 2.5 h (75°C)	TaKaRa Biomedicals
Carboxypeptidase	*S. solfataricus*	C-terminal sequencing	Broad specificity (can release basic, acidic, and aromatic residues); stable in solvents at 40°C	66
Alkaline phosphatase	*T. neapolitana*	Enzyme-labeling applications where high stability is required	Optimal activity at 85°C, pH 9.9; $t_{1/2}$, 4 h (90°C) (+ Co^{2+})	87

Methanogens

- **Methanogens** (methane producing microorganisms)
- Methanogens belong to the domain archaea
- There are over 50 described species of methanogens,
- Methanogens are usually either coccoid (spherical) or bacilli (rod shaped).

Methanopyrus kandleri: a methanogen, only strain in the genus Methanopyrus can survive and reproduce at 122°C.



Classification of Methanogenic Bacteria

Based on the convertion of substrate like, acetic acid, H_2/CO_2 and formate and methanol, methylamine or dimethyl sulfide into methane and CO_2, Methanogens can be divided into three groups:

1. Hydrogenotrophic - H_2/CO_2
2. Aceticlastic, and - acetic acid
3. Methylotrophic methanogens - methanol and methylamine/ dimethyl sulfide

Sl.No.	Group	Substrate	Representative species
1.	MB I	Acetate	*Methanosaeta* spp.
2.	MB II	Hydrogen and Formate	*Methanobrevibater* spp. *Methanogenium* spp.
3.	MB III	Methylated Compounds	*Methanolobus* spp. *Methanococcus*
4.	MB IV	Acetate, H_2 & Methylated Compounds	*Metahanosarcina* spp.

Habitat of methanogens

- Methanogens inhabit in some of the most extreme environments on earth,
- Rumen of ruminants living on H & CO_2 produced by syntrophic microbes that help digest cellulose, as well as necessary for protein synthesis.
- Muck of swamps and marshes, hydrothermal vents, porous rock, sewage sludge, termite-gut and oil contaminated groundwater at underground oil storage facilities.

- Based on their natural habitat, some are **thermophiles**, the methanogens found in **volcanic hot springs, mud volcanoes and solfataras**, where temperatures span from **40–100°C** and,
- in marine environments in undersea hydrothermal vents where the temperatures can reach up to **350°C** due to high pressure.
- Psychrophily is rare among methanogens with only a few species being identified till now….
- Also, there are halophiles, acidophiles, alkaliphiles, radophiles, and so on.

Applications of methanogens

Hydrogen production. It has been observed that several methanogenic strains can also produce H - *Methanothermobacter marburgensis, Methanosaeta thermophila*.

Biogas production from organic matter: Butyrate, propionate, acetate, formate, ethanol, H_2 and CO_2..

Treatment of sewage water: breweries, paper mills, oil mills, dairy production and others…

Treatment of solids: Biodegradable wastes like animal manure and slurry from the production of pig, poultry, fish and cattle .

Micro biogas systems: Domestic organic waste or feces, while the produced gas can be used directly for heating and cooking.

- **Bioelectrochemical systems:**
- **Removal of organic pollutants** in **aquatic bodies.**
- **Deazaflavin coenzymes:** Serve an vital role in the conversion of CO_2 and of low molecular weight organic acids into methane. In streptomycetes, they function as antenna chromophore of DNA photolyase.
- Methanogenic archaea play an essential role in the **reintroducing unavailable C to the C-cycle** by anaerobically.
- The methanogens are used **to break down this cellulose.**

Global warming. Methanogenic Archaea **producing methane gas, a potential fuel source as well as a greenhouse gas.**

Promoting digestion: Methanogens of human digestive tract play major roles, in particular the use of H from the fermentation products of bacteria.

References

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REVIEW ARTICLE

Insight into thermophiles and their wide-spectrum applications

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Anil K. Sharma[1]

CHAPTER 1

Extremozymes and their applications

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Hyperthermophilic Enzymes: Sources, Uses, and Molecular Mechanisms for Thermostability

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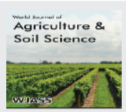

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Review Article

Review on Methanogenesis and its Role

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