Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Research Article

Received on: 18-01-2016
Accepted on: 21-01-2016
Published on: 26-01-2016

Winogradsky column and Microbial Metabolic Network Analysis

Santosh Kumari Duppala*, DSVGK Kaladhar

Corresponding Author:

*** Santosh Kumari Duppala,**
Department of Microbiology,
Bilaspur University,
Bilaspur- 495001,
Chhattisgarh,
India.

Email: dsantoshkumari87@gmail.com

ABSTRACT

The Winogradsky column is mainly constructed to know that how the microbial diversity and ecological relationship between the microorganisms exit in their natural environment. In the present review, by using Winogradsky column the microbial metabolism and its network analysis is investigated. Prokaryotes and Archaea bacteria show great metabolic diversity and these microorganisms keeps our biological world continuous recycling. Different types of organisms are detected in this present study; Winogradsky column forms three layers according to their energy and light requirement. On the top layer majority of the microbes in the water layer were photoautotrophic organisms like *cyanobacteria, Algae,* and many sheathed bacteria and chemoautotrophs such as Beggiatoa and Thiobacillus sulfur oxidizing bacteria are dominant on surface but the ratio decreases as in the lower layers .Further, the next gradient promote aerobic chemoheterotrophic bacteria are more in number in the higher or middle layer of the column when compared to bottom –green and purple sulfur phototrophic bacteria like *Rhodhospirilum, Rhodhomicrobium, Rhodhopseudomonas and anaerobic Chlorobium* are found in the microbial population. In the oxygen depleted zone or bottom layer anaerobic chemoheterotrophic or sulphur reducing bacteria are found more dominant such as Desulfovibrio and clostridium are mostly found. Phylogenetic tree was constructed using MEGA v7 Software.

Key-words: Winogradsky column, layers, microbes, phototrophic bacteria, chemoheterotrophic organisms, microbial ecology.

Cite this article as:
Santosh Kumari Duppala, DSVGK Kaladhar, Winogradsky column and Microbial Metabolic Network Analysis, Asian Journal of Pharmaceutical Technology & Innovation, 04 (16); 2016, 74-83. www.asianpharmtech.com

INTRODUCTION:

A Russian microbiologist named Sergai Winogradsky worked in 1800's -1900's on Environmental microbiology, Ecology; he is one of the great founders of microbiology. He constructed a simple device called Winogradsky column this allows us to see the microorganisms in the microbial ecology. The main purpose of this column is to observe the growth and development of microorganisms in the microbial ecology.

Winogradsky column it is a glass container anoxic device that is used as a long term inoculant for enrichment cultures it is particularly good for the enrichment cultures of purple, green phototrophic bacteria and sulfate reducing bacteria.

The Winogradsky column is made of tall glass or plastic tube and it is filled with one third of river or lake mud is collected (while collecting the mud remove all the stones, sticks, pebbles, debris) and water is added to make uniform slurry. To that slurry nutrients are supplemented like carbon and sulfur. Carbon source such as hay, shredded newspaper (contains cellulose), blackened marshmallows or eggshells contain calcium carbonate and sulfur source such as calcium sulfate or egg yolk are added and make a slurry of mud and nutrients .Rest of the tube is filled with water from river and tube is capped tightly and kept for incubation near the window for sunlight.

After 3 to 4 months later different types of microorganisms occupy distinct layers showing aerobic, anaerobic, and sulfide zones in the column

Figure1: Winogradsky column

MATERIALS USED:

- Plastic wrap
- Rubber band
- Plastic bottle or glass tube,
- River mud
- Egg yolk, (for calcium sulphate)
- Shredded newspaper (for cellulose)
- Egg shells and blackened marshshallows (calcium carbonate)
- River water

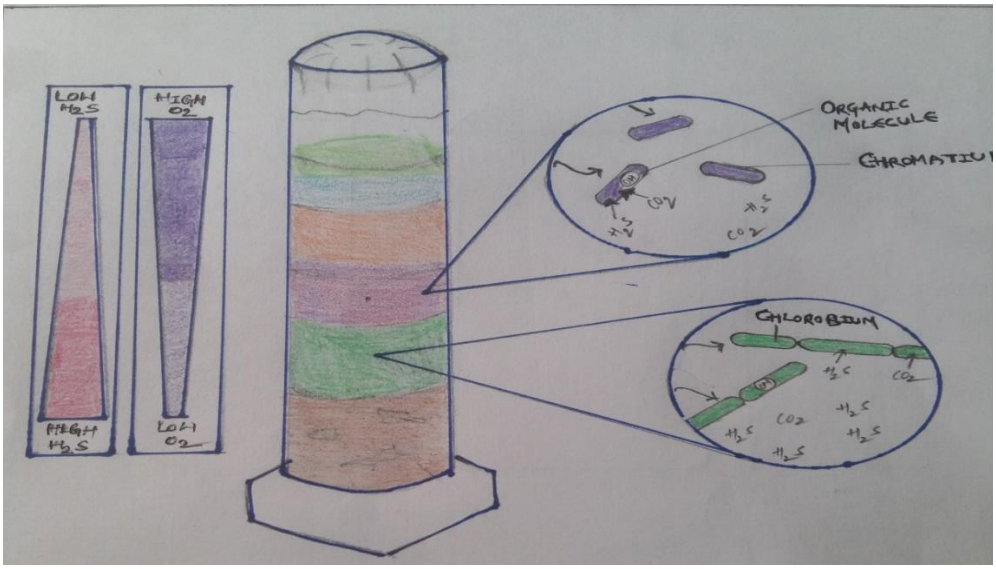

Figure 2 : oxygen, sulfur, sunlight gradients
After 3 to 4 months later different types of microorganisms occupy distinct layers showing aerobic, anaerobic, and sulfide zones in the column

Winogradsky column shows different coloured bands showing photosynthetic bacteria in the column. These forms mainly three zones :

1. Aerobic
2. Micro aerophilic
3. Anaerobic zone

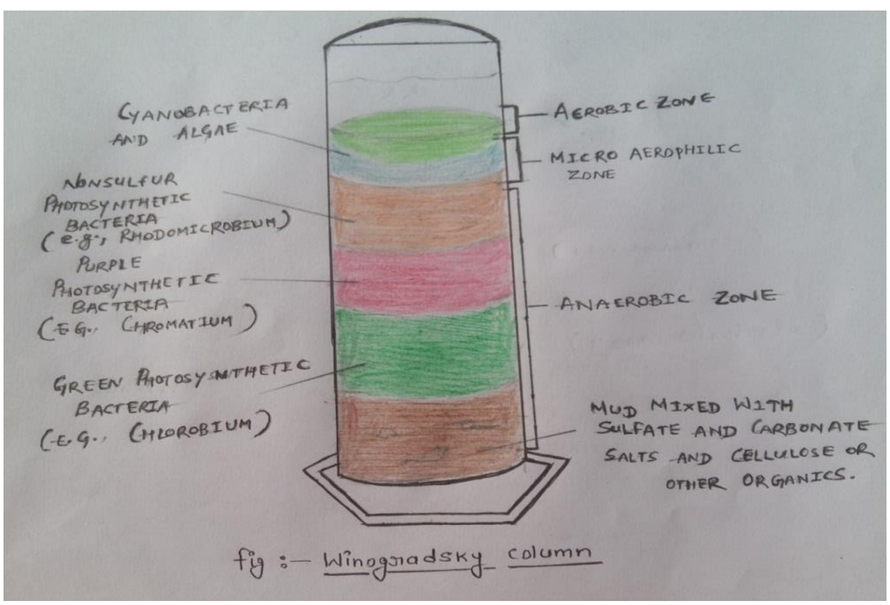

Figure 3 : Column showing microbial network and colored bands

Aerobic:

On the top of the oxygenated zone there exist different aerobic organisms, Algae and Photosynthetic cyanobacteria shows green color. They are formed by spiral filaments and there is a strong belief that chloroplasts plants originated as Cyanobacteria.(They are nitrogen fixing bacteria) .Cyanobacteria is also known as blue-green algae and are valid for compatible systematic terms. This micro-organism contains both unicellular to multicellular prokaryote that contains chlorophyll a and performs oxygenic photosynthesis related with photosystems. Their life processes require only carbon dioxide, water, inorganic compounds and light.

$$CO_2 \text{-------} \; CH_2O; \; H_2O\text{--}O_2$$

$$CH_2O \text{------} \; CO_2; \; O_2\text{--}$$

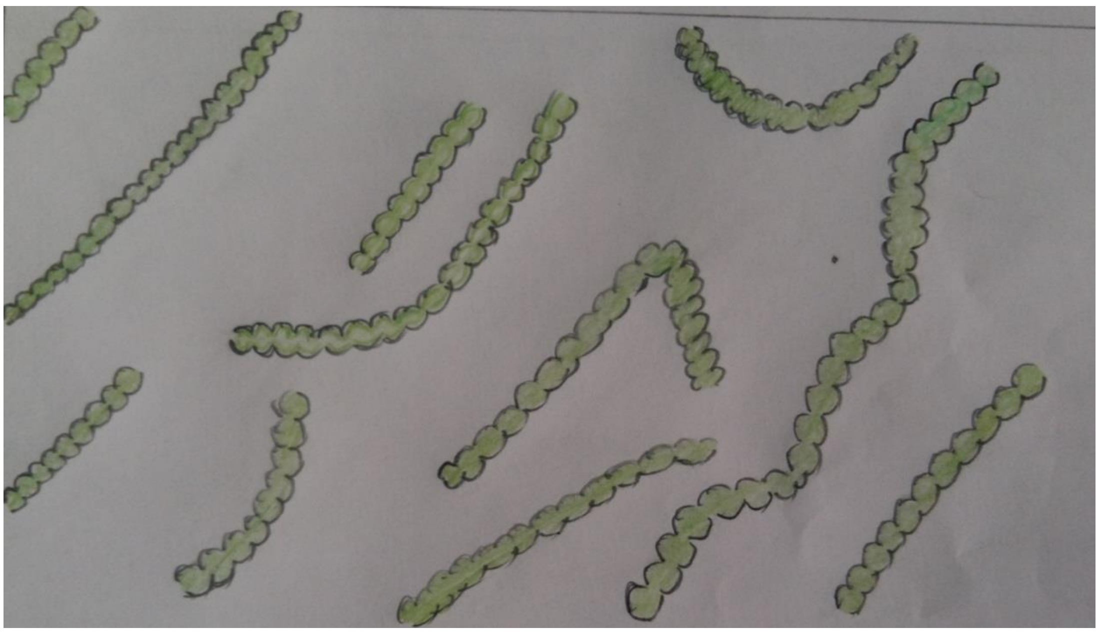

Figure 4: Cyanobacteria

SULPHUR OXIDIZING BACTERIA:

H_2S from bottom layer diffuses into aerobic gradient where it is oxidized to sulphate by Sulphur oxidizing bacteria. These organisms termed as Chemoautotrophs – Beggiatoa and Thiobacillus oxidize sulfate.

Beggiatoa forms long filaments hairs like structures and it is mainly used in Winogradsky classic experiment – 'autotrophy', it comes out of the mud at night, at night H2S increases and comes out for low levels of O2.

$$H_2S + 2O_2 \rightarrow SO_4^{=} + 2H^+$$

$$HS^- + 1/2\, O_2 + H^+ \rightarrow S^0 + H_2O$$

$$S0_2 + H_2O + 2O_2 \rightarrow 2SO_4^{=} + 2H^+$$

Figure 5 : Beggiatoa

Micro-aerophilic:

In this zone oxygen is scarce and it diffuses down, whereas Sulfur from lower zone moves up in the form of H2S. In this column anaerobic photosynthetic bacteria can grow. Purple Sulfur bacteria and green sulfur bacteria are normally distinguished by two narrow bright coloured bands immediately one above the sediment layer.

GREEN AND PURPLE SULPHUR BACTERIA:

Green sulfur bacteria have smaller cells like chlorobium is characterized by green colour and grows in anaerobic conditions.

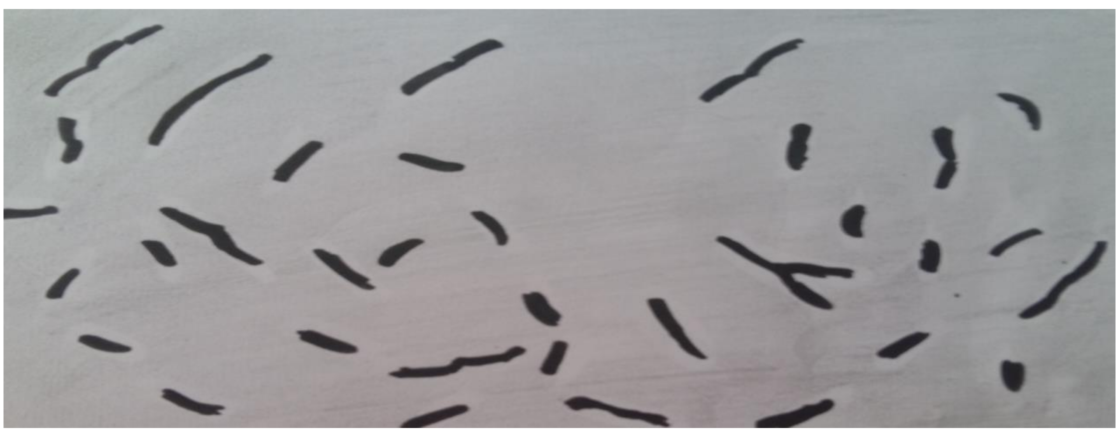

Figure 6 : Chlorobium

In the microaerophilic zone such as *Rhodospirillum and Rhodopseudomonas* appears in light orange colour and they have large cells they deposit sulfur granules inside the cells.

$H_2S = S^0$, $CO_2 = CH_2O$

$H_2 = S^0$, $CO_2 = CH_20$

Above the photosynthetic bacteria purple non-sulfur bacteria shows bright red colour include species Rhodopseudomonas, Rhodospirillum and Rhodomicrobium.

$$6CO2 + 6H2O \rightarrow C6H12O6 + 6O2 \text{ (Plant photosynthesis)}$$

$$6CO2 + 6H2S = C6H12O6 + 6S \text{ (Bacterial anaerobic photosynthesis)}$$

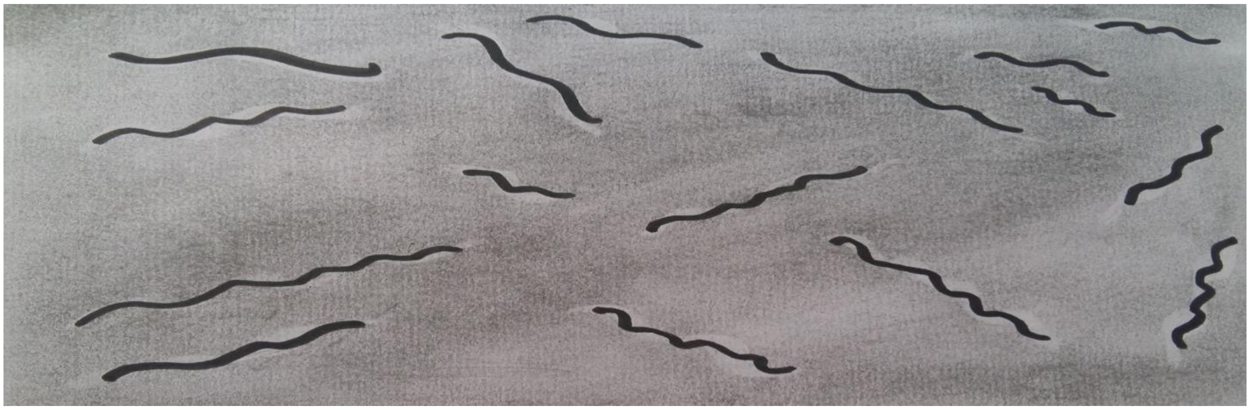

Figure 7 : Rhodospirillum

Anaerobic Zone :

In this gradient only those organisms that are able to ferment organic matter and grow in the absence of O2 anaerobic respiration perform.

SULPHUR REDUCING BACTERIA:

Sulfur reducing bacteria such as *desulfovibrio* perform anaerobic respiration they are also considered as obligately anaerobic, aerotolerant species. During the metabolism of sulphate reducing bacteria, the sulfate is reduced to hydrogen sulfide (H2S), with combines with Fe+2 and forms FeS, this sediment appear black.

H_2S+Fe = FeS + H_2

Clostridium species (cellulose-degrading) bacteria are grows when the oxygen is completely depleted in the sediment. Clostridium species are anaerobic because their vegetative cells are eradicate by exposure to oxygen, but they can survive as spores in aerobic conditions. They reduce the cellulose to glucose and then ferment the glucose to produce energy. They are obligate anaerobes which produce endospores and they are bottle shaped.

So_4^{2-} = H_2S, CH_2O = CO_2

CH_2O= CO_2 =H_2

Figure 8 : Desulfovibrio

Table 1: Microbial Environment

Oxygen Level	Sulfur Level	Gradients	Possible Organisms	Observations	Environment	References
High Oxygen	Low Sulfur	Air	Algae		Sunlight	Stewart,w.d.p
Decreasing oxygen	Increasing Sulfur	Aerobic Zone	Sheathed Bacteria	Grass green		
			Cyanobacteria, Beggaiota, Thiobacillus, diatoms, Protists		Decreasing Sunlight	Pipage,Helen K
		Microaerophilic Zone	Photoheterophs Purple non-sulfur Bacteria	Brownish - Red		Sagan , Dorion
			Purple Sulfur Bacteria	Purple	Low Sunlight	Zavarin
			Green Sulfur Bacteria	Green		
Low Oxygen	High Sulfur content	Anaerobic sediment	Sulfur reducing bacteria	Gray		Madigan M;Martinko
			Clostridium	Black		

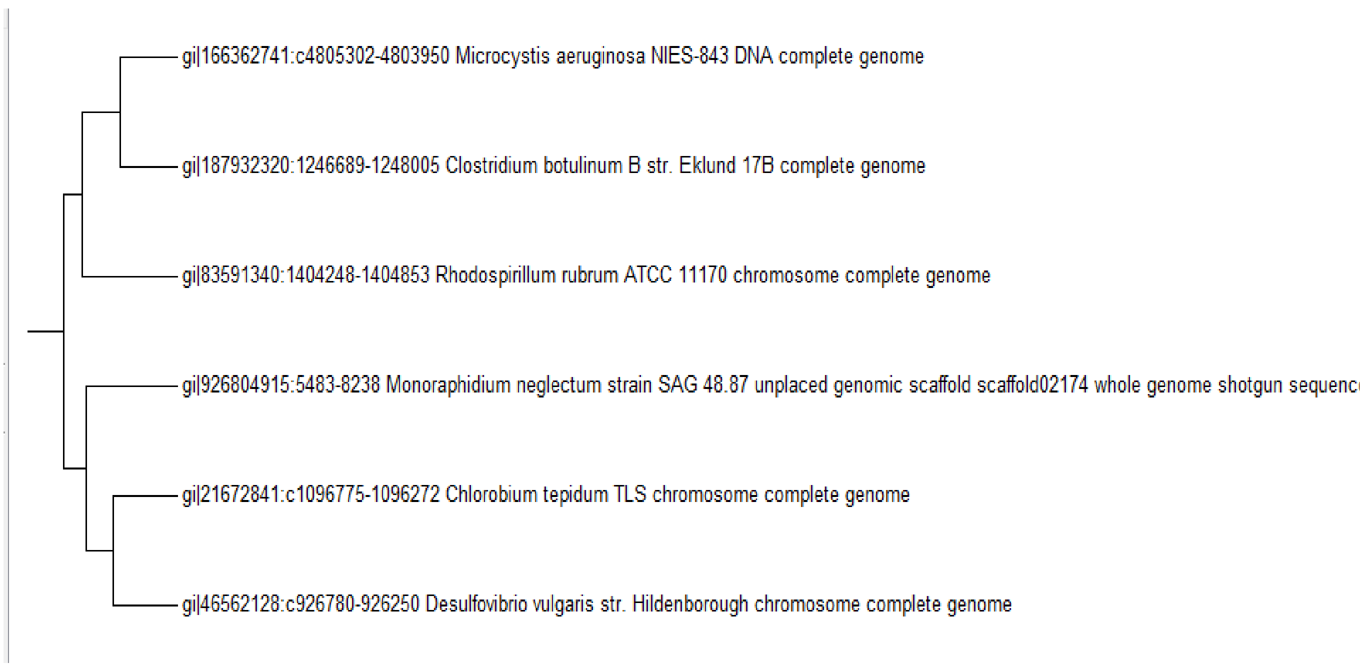

Figure 9: Phylogenetic tree (UPGMA) using MEGA

The phylogenetic tree is constructed by performing microbial column assessment using MEGA. On sequencing 16srRna the Phylogenetic tree shows different microorganisms the species Clostridium, Microcystis aeruginosa belongs to same group and also Rhodospirillum occupies the same ancestor of bacteria. Then the group Chlorobium tepidum, Desulfovibrio vulgaris, belong sulphur reducing bacteria and monorphidium neglectum belong to algae

References

1. Alexander, M. (1981). Biodegradation of chemicals of environmental concern. Science, 11,132-138. http://dx.doi.org/10.1126/science.7444456
2. NASA- National Aeronautics and Space Administration (2001). Building a Winogradsky Column. [Online] Avaliable at: <http://quest.nasa.gov> (April 20, 2012).
3. *Pellegrin, Véronique; Juretschko, Stefan; Wagner, Michael; Cottenceau, Gilles (1999).* "Morphological and Biochemical Properties of a Sphaerotilus sp. Isolated From Paper Mill Slimes" *(PDF).* Applied and Environmental Microbiology *65 (1): 156–62.* PMC 90997*.* PMID 9872774. Retrieved September 26, 2012*.*
4. Zavarin, G.A. (2006). Winogradsky and Modern Microbiology. Microbiology. (75)3, 501-511
5. Pigage, Helen K. "The Winogradsky Column: A Miniature Pond Bottom." American Biology Teacher (47/4), April, 1985. Pp. 239-240.
6. Sagan, Dorion, and Margulis, Lynn. Garden of Microbial Delights. Boston: Harcourt, Brace, Jovanovich.1988
7. Mitsui, A., Takahasi, A., Ikemoto, H., Cao, S. & Arai, T. 5th Int. Symp. Photosynth. Prokaryotes, Abstr. 63 (Grindelwald, Switzerland, 1985).
8. Mullineaux P.M, Chaplin, A.E. & Gallon, J. R.J. gen Microbiol. 120, 227-232(1980).l ISI l ChemPort l