**Winogradsky Column (Theory)**

Winogradsky column begins as a uniform slurry of soil, mud or lake sediment and water supplemented with nutrients,(carbon and sulfur).

The ingredients are mixed into a uniform slurry, poured into a tall, transparant vessicle such as a graduated cylinder or soda bottle and exposed to sunlight to provide energy for the system and to enrich for both aerobic and anoxic photosynthetic bacteria.

The sediment, soil and water serve as inocula of diverse microbial populations with diverse metabolic processes.

A series of gradients (e.g. oxygen, H2S) soon develop in the column as a result of

bacterial metabolic processes.

For example, aerobic photosynthetic organisms and exposure to the air will produce O2 near the top of the column which can then be used by other microbes for aereobic respiration.

Similarly, sulfate reducers in the anoxic region near the bottom of the column will produce H2S, which can be used by H2S utilizers and anoxic phototrophs.

*The term* ***anoxia*** *means a total depletion in the level of* [*oxygen*](http://en.wikipedia.org/wiki/Oxygen)*, an extreme form of hypoxia or "low oxygen".*

Different organisms soon gain a competitive advantage in different regions of the column causing a stratification and continual regional succession of organisms.

Because this culture system more closely resembles a natural environment

than do traditional culture methods, a wider variety of physiological types can be

observed than by the more standard culture methods.

Also, different microbes will be concentrated (enriched) in specific regions of the column where local conditions are optimal for their particular growth (e.g areobes at the top in the oxic zone and anaerobes at the bottom, anoxic zone).

Winogradsky columns demonstrate the interdependence of diverse microorganisms in

complex communities for survival and growth. As such, variations in Winogradsky column ingredients and manipulations are as infinite as our imagination.

Microbial process could be studied with the correct design and initial inocula.

**One Day Prior to Building the Column: (for teacher)**

1. Gather mud or sand from a forest, garden, lake, pond, marsh, or ocean.

**Extension:** If possible, gather mud or sand from a variety of places. Columns can be set up representing

each place the mud or sand was collected from. This will allow the students to compare the microbial

growth from a variety of places. For example, if some of the mud was from a freshwater area while

some was from a saltwater area, a comparison could be made about the amount of microbial growth.

2. Gather water from each mud or sand location used.

3. Carefully cut off the top of the 2-liter bottle to use as a funnel.

4. Use a pencil sharpener to powder the chalk. Depending on grade level and resources available,

students can do

this step while making their column.

5. Use a mortar and pestle to mash the hard-boiled egg yolk. Depending on grade level and resources available,

students can do this step while making their column.

6. Set out materials for each group.

**Directions for Building the Column: (for students)**

1. In a small bucket, add 5 cups of mud or sand. Remove any sticks, leaves, or rocks.

2. Stirring the mud or sand with a large spoon or paint stirrer, slowly add water until the mixture is like thick cream.

Be careful not to add too much water.

3. Shred a full sheet of newspaper into very small pieces. Add the newspaper shreddings to the mixture.

4. Then add 1 tablespoon of powdered chalk to the mixture.

5. Add 1 teaspoon of mashed hard-boiled egg yolk or calcium sulfate to the mixture.

6.Stir the mixture gently using a large spoon or paint stirrer. Make sure the mixture is fluid so it will flow through the funnel.

7. Remove any labels from your bottle. Make a new label with the names of the students in your group as well as the

source of the mud or sand.

8. Set the funnel into the mouth of the bottle. Secure the funnel with tape or have a group member hold the funnel

in place.

9. Pour or scoop a small amount of the mixture into the base of the bottle.

10. Place your hand over the top of the bottle and tap the bottom of the bottle firmly on the table. This helps the

mixture settle and removes oxygen that is trapped in the mixture.

11. Repeat the two previous steps of adding a small amount of mixture and settling the mixture until the bottle is about 90 percent full.

12. Stir the mixture in the bottle to remove any air bubbles.

13. Let the bottle sit for 30 minutes. The water that settles on top of the mixture should

be about 2 cm deep. Add/remove the water in your bottle as needed.

14. Cover the bottle with foil or plastic wrap and a rubber band.

**Extensions:**

If multiple columns are made, place half of the columns placed in the dark. This will allow the students

to compare microbes that require light with those that do not.

Note to teacher: Depending on grade level, a discussion on photosynthesis could be conducted at this point. Photosynthesis is the

process by which living things such as plants make their own food by using energy from the sun. Students in the older grade levels

can be introduced to the concept that without the photosynthetic activity of early bacteria, Earth’s atmosphere would still be

without oxygen.

Materials can be gathered from saltwater or freshwater sources. If you have access to both, half of

the columns could be made with materials from freshwater sources while the other half could be made

with materials from saltwater areas.

15. Once columns are completed, have students re-read their hypotheses and decide if they want to make any changes

to it. Record this modified hypothesis in the lab journal.

Note to teacher: Scientists would not modify their hypothesis after beginning the investigation, but due to the students’ limited knowledge on

the subject matter, it may be helpful.

**Extension:**

Depending on time and grade level, the students could be asked to make more specific hypotheses. For

example, the students could develop hypotheses to these questions:

• Will sunlight have an effect on the column?

• Will freshwater or saltwater allow more microbial growth?

• Will microbes be able to grow better at the surface of the column or at the bottom of the column?

Encourage students to provide reasoning behind their hypotheses.

**Winogradsky Column:**

**Collecting data:**

1. Mark the level of the mud-water interface on your column using a permanent marker. Make this the zero-level.
2. Starting from the zero level, mark off 3 cm levels till the top.
3. Sample water from each of the level.
4. Close the free end of a 1ml pipette with a finger and insert the pipette into the column upto the right depth. Release the finger and allow about 0.1 ml of water to get into the pipette.
5. On a clean glass slide, let one drop fall on the slide. Observe the slide under a microscope and note down the type and number of organisms seen. Repeat 3-5 times for each level to get a good estimate within each level.
6. Repeat for all levels.
7. Repeat this activity once every week.
8. Come up with a data sheet which shows the details of your data collection. This data sheet should be approved by your instructors.

***8/03/2013-*** Winogradsky column observations

**a) NaOH ( 10X magnifications)**

*Top Layer*- A lot of sand particles

*Middle layer*- smaller parameciums and baby parameciums also

*Bottom layer*- Smaller than the lab parameciums.

b) **Urea ( 4X magnification)**

*Bottom layer-* A lot of sand particles. No moving things

Middle layer- thread like structures, lots of sand

*Top layer*- Sand particles, No moving organisms.

c) **Control(10X magnification)**

1) Bottom layer- Silica as dark colored big pieces, light colored stationary structures can also be seen.

2)Bubble like tiny organisms can e seen moving very fast underneath the food materials, thread like structures of light green color can also be seen. The thread like structures looks like plant roots.

3)Bubble like organism which are a lot smaller than the parameciums can be seen.

4)Moreover, an organism with a tail, which looks more like a wine glass shaped one. This wine glass shaped organism looks transparent, but it can also change its shape.. becomes circular, wine glass shaped and so on..

5)It tends to move along a straight line. We did not see any organism to organism interaction amongst these..

*Middle layer*- Silica, bubble like organisms, drop shaped transparent organisms moving very fast (but only one) can also be seen.

*Top Layer*- Some structures that appear like plant root, silica could be seen.

**EGG YOLK( 10X Magnification)**

*Top Layer-*

1)Lots of silica, oval organisms which are transparent and they move in circles are seen. Their motion is like, moves in circles and then stays, and so on..

2) Other smaller bubble like fast moving organisms can also be seen.

*Middle Layer-*

Silica plus same as the top layer.

*Bottom Layer*-

1) Silica, same as top layer, plant like or some stuff that looks like moss.

2)We say plants because of the thread like appearance when compared with silica crystals.

**25/03/2013**

**NaOH(10X Magnification)**

*Top layer*- Small sized parameciums, small transparent organisms that seems to have contaminated the cultures.

Middle layer- Nothing

Bottom Layer- small parameciums.

**CONTROL**

**Top Layer**- Nothing

**Bottom layer**- Nothing

Middle layer- Nothing

**EGG YOLK**

Nothing

**UREA**

Nothing

***18/03/2013*** – Winogradsky Column Observations

a) **Egg Yolk**- (Magnification 10X)

Zero Level

Some silica, transparent membranous structures, tiny bubble like structures floating about.

First Level

A lot of silica, some ciliated transparent structures with dog tail kind of motion.

Second Level

Contains silica, very fine hair like structures, some ciliated transparent organisms

Third Level

Same as the second level observation.

b**) Control** –(Magnification 10X)

Zero level

Silica, much extremely small bubble like structures.

First Level

Same as the zero level.

Second level

Silica and a large number of ciliates and round organisms.

Third level

Same as the second level.

c) **NaOH**- (Magnification 10X)

Zero Level

Silica and extremely tiny round organisms.

First Level

Slightly big black round organisms, silica and extremely tiny round organisms.

Second Level

Silica and many huge ciliates

Third Level

Extremely fast moving ciliates, very tiny round organisms and a lot of silica.

**Urea**-(Magnification 10X)

Zero Level

Silica and fine transparent hair like structures

First level

Silica, fine transparent hair like structures, small tiny transparent bubble like structures

Second Level

Same as the first level

Third level

Only Silica and nothing else

**3rd March 2013**

**EGG YOLK**

**Zero Level**

Silica crystals, some plant moss like structures, very tiny black dot like organisms.

**First Level**

Contains silica crystals

**Second Level**

Lots of mud and silica crystals.

**UREA**

**Zero level**

No mud, silica. No organisms present.

**First level**

No mud, silica. No organisms present.

**Second level**

Contains silica .

**NaOH**

**Zero Level**

No mud, silica. No organisms present

**First Level**

No mud, silica. No organisms present.

**Second level**

Contains silica

**CONTROLL**

**Zero Level**

No growth, appears greenish, Nothing ele present.

**First Level**

Very little silica present

**Second Level**

Silica crystals, some plant moss like structures.

**8th April 2013**

Naoh –

middle- 10x small paramecium ,rest silica

Egg Yolk –

top –

10x small transparent spherical organisms,rest silica

Control

empty except silica

Urea –

empty except silica

**17th March 2013**

**UREA**

Nothing

**NaOH**

Zero Level

Same organisms as the second level.

First Level

Nothing

Second Level

Only one type of organism. It is very small under 4X, has an oval shape and travels at a moderate speed, with respect to paramecium.

Third Level

Same organisms as in second level. Nothing Else.

**CONTROL**

Nothing

**EGG YOLK**

Nothing