

MICROBIOLOGY
EXERCISE 5
Metabolic Activities of Bacteria

Group Members	Name:	Rating:	PTS:
Group Number _____		Date Performed:	
		Date submitted	

I. OBJECTIVES:

1. Learn how to use differential and selective media to identify bacterial species.
2. Perform the following biochemical tests and understand how they work: carbohydrate fermentation, casein hydrolysis and citrate utilization
3. Relate the results of the tests performed to the different types of metabolic activities carried out by individual bacterial species.

II. MATERIALS:

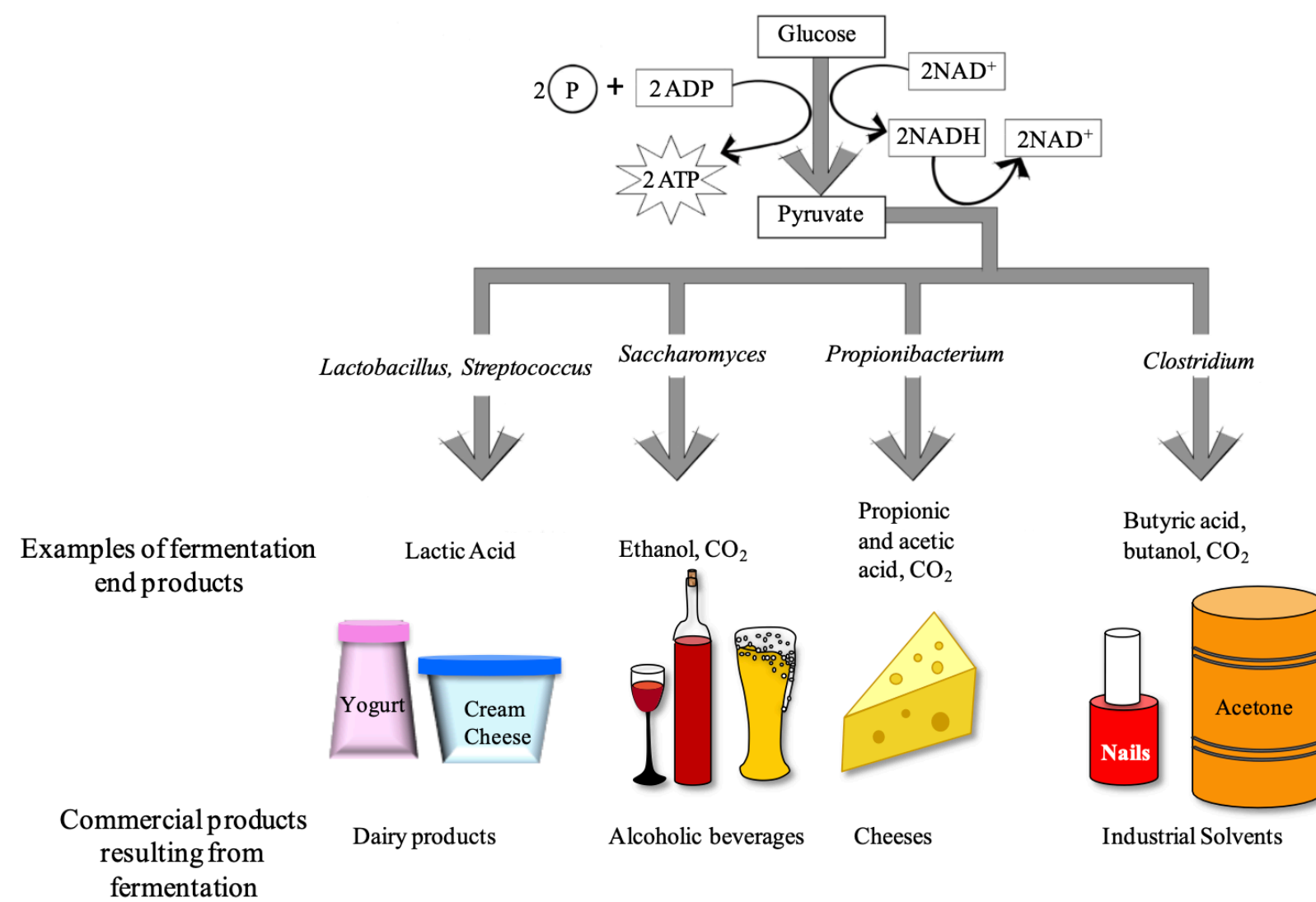
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|--|----------------|
| Simmon's Citrate Agar | Test tubes |
| Skim Milk Agar | Petri Dish |
| Glucose | Nutrient Broth |
| Sucrose | Peptone |
| Lactose | |
| Durham tubes | |
| Culture (<i>E. coli</i> , <i>S. aureus</i> and <i>P. vulgaris</i>) | |

A. Carbohydrate Fermentation

Fermentation is a metabolic process that some bacteria use to break down glucose when O₂ is not available. Fermentation includes the reactions of glycolysis (where a single molecule of glucose is broken down into 2 molecules of pyruvate), as well as additional reactions that produce a variety of end products (acids, alcohols, gases). The end products are characteristic of individual bacterial species.



Figure 1. Carbohydrate Fermentation



Although the ultimate substrate molecule for fermentation is always glucose, some bacteria use additional chemical reactions to convert other monosaccharides as well as disaccharides into glucose. Therefore bacteria can be differentiated both based on their ability to ferment various carbohydrates, as well as the end products that result from the fermentation process. The medium used to test carbohydrate fermentation is a nutrient broth that contains a fermentable carbohydrate (usually a monosaccharide or a disaccharide), peptone (amino acids) as well as a pH indicator. The pH of the medium is adjusted to approximately 7.5, so it appears orange/red when using phenol red. If the carbohydrate in the medium is fermented and acidic end products are formed, a color change to yellow will result. Occasionally, bacteria will not ferment the carbohydrate, but instead will break down proteins producing ammonia (NH₃) in the growth medium. In this case, the medium will become more alkaline.

Some bacteria will produce gases when fermenting a carbohydrate. To detect these gases, a **Durham tube** is used. This is a small inverted tube that is placed within the larger glass tube containing the fermentation medium (see below). If gases (typically CO₂) are produced during the fermentation process, a bubble will form at the top of the Durham tube. If you see a bubble in the Durham tube, the medium will also be acidic.

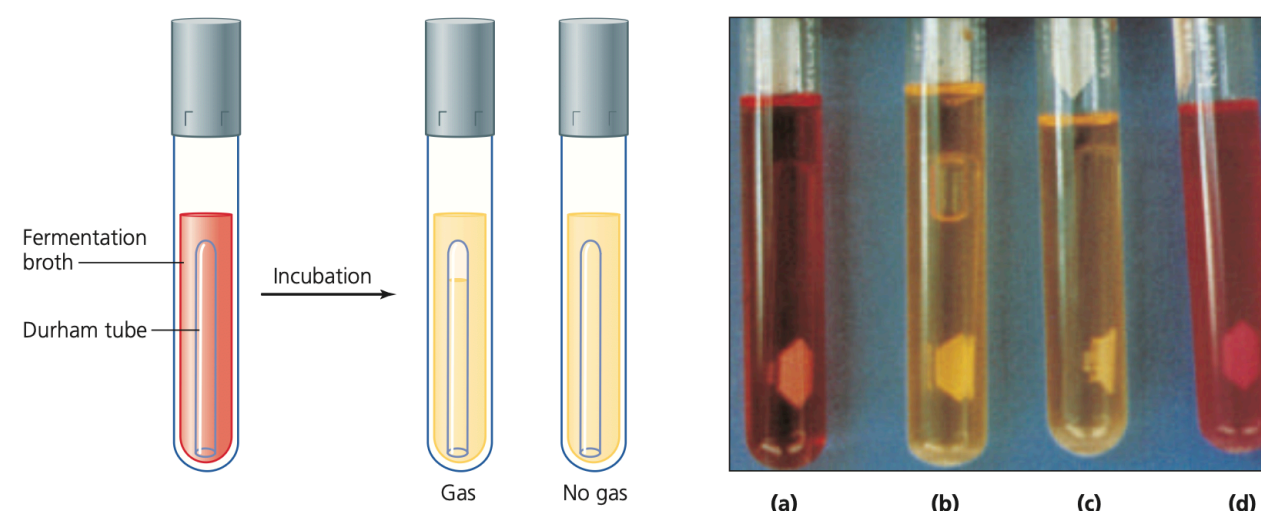
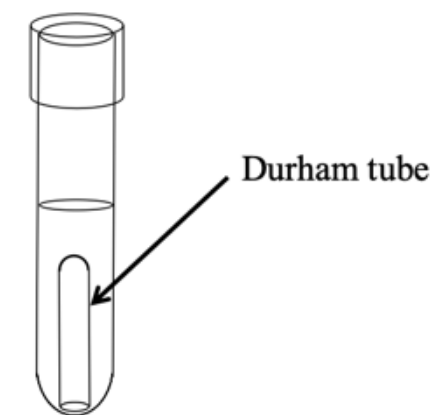


Fig 2. Detection of gas production. Fig 3. Carbohydrate fermentation test.
(a) Uninoculated, (b) acid and gas, (c) acid, and (d) negative.



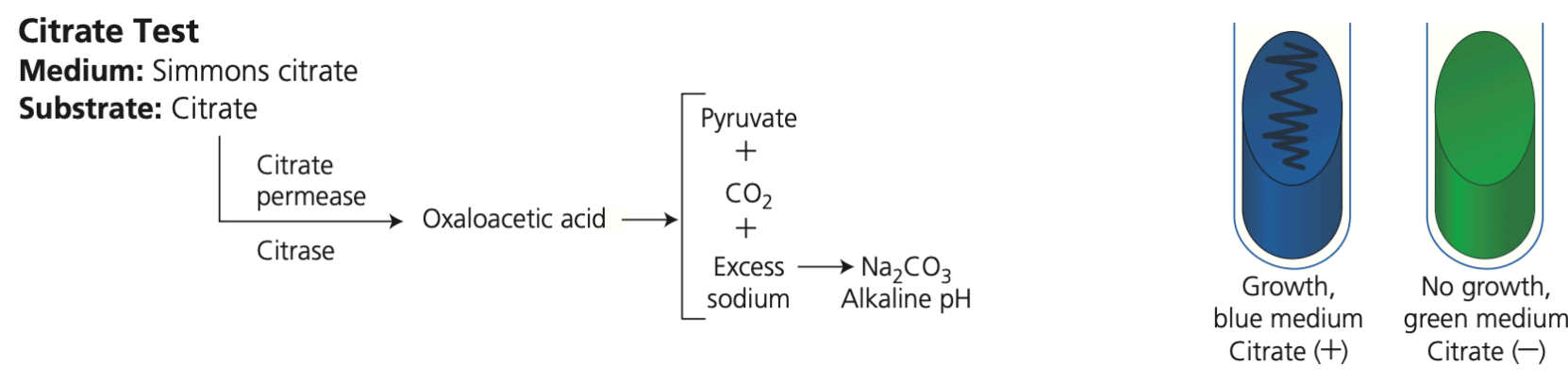
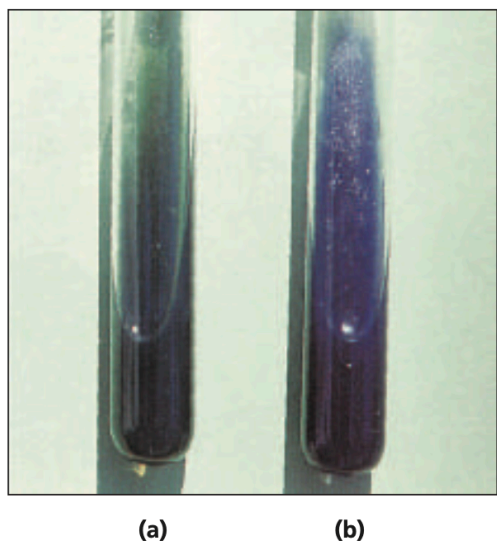
B. CASEIN HYDROLYSIS

Some bacteria secrete enzymes called proteinases that break down proteins. You have already read about one kind of proteinase—gelatinase. Milk contains large proteins called **caseins**. Some bacteria secrete **caseinases** that break down casein outside of the bacterial cell so the smaller products (e.g., amino acids) can be transported inside the cell and further metabolized.

Milk agar (which contains powdered milk) is used to detect the presence of bacterial caseinases. This medium is cloudy because when milk is mixed with agar, the casein forms a colloid through which light cannot pass. The presence of caseinases can be detected by observing a clearing in the agar around the bacterial growth, which indicates that the caseins have been broken down into transparent end products (amino acids and peptides), which are then taken up by the cells.

C. CITRATE UTILIZATION

Organisms that can survive using citrate as the sole source of carbon have a **citrate permease** enzyme that can transport citrate molecules into the cell. The citrate is then made into pyruvate, which can be converted into a variety of different products. **Simmon's citrate** is a chemically defined medium that contains sodium citrate as the sole carbon source as well as the pH indicator bromothymol blue. Bacteria that can grow on this medium (i.e., that can survive on citrate as the sole source of carbon) produce alkaline byproducts that will change the medium from green (neutral pH) to blue (alkaline pH).



Citrate utilization test.

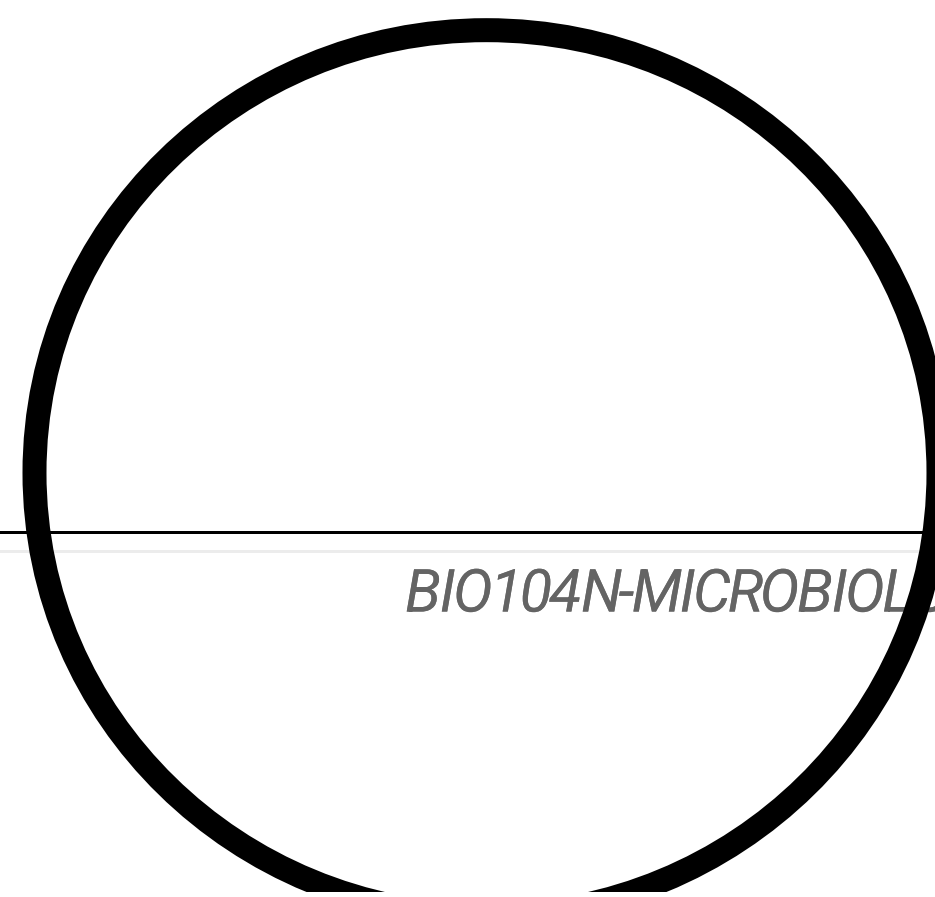
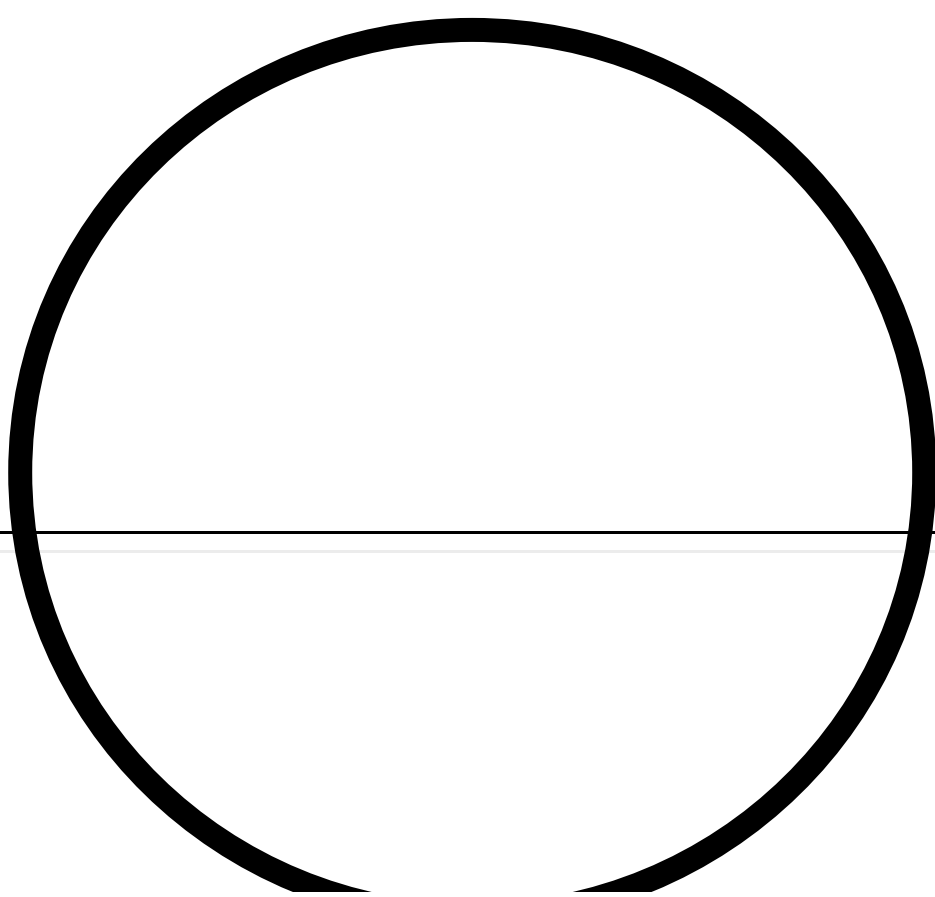
- (a) Tube is negative, showing no growth on slant surface.
 (b) Tube is positive, showing growth on slant surface.

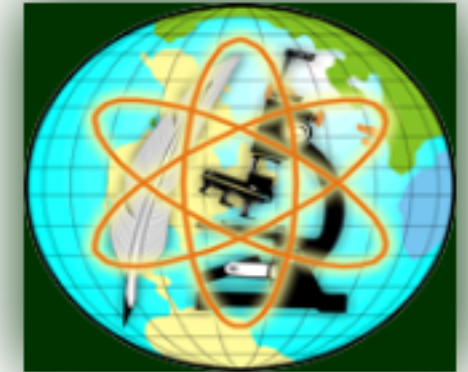
IV. DATA AND RESULTS

A. CARBOHYDRATE FERMENTATION

Actual Specimen

Researched Specimen



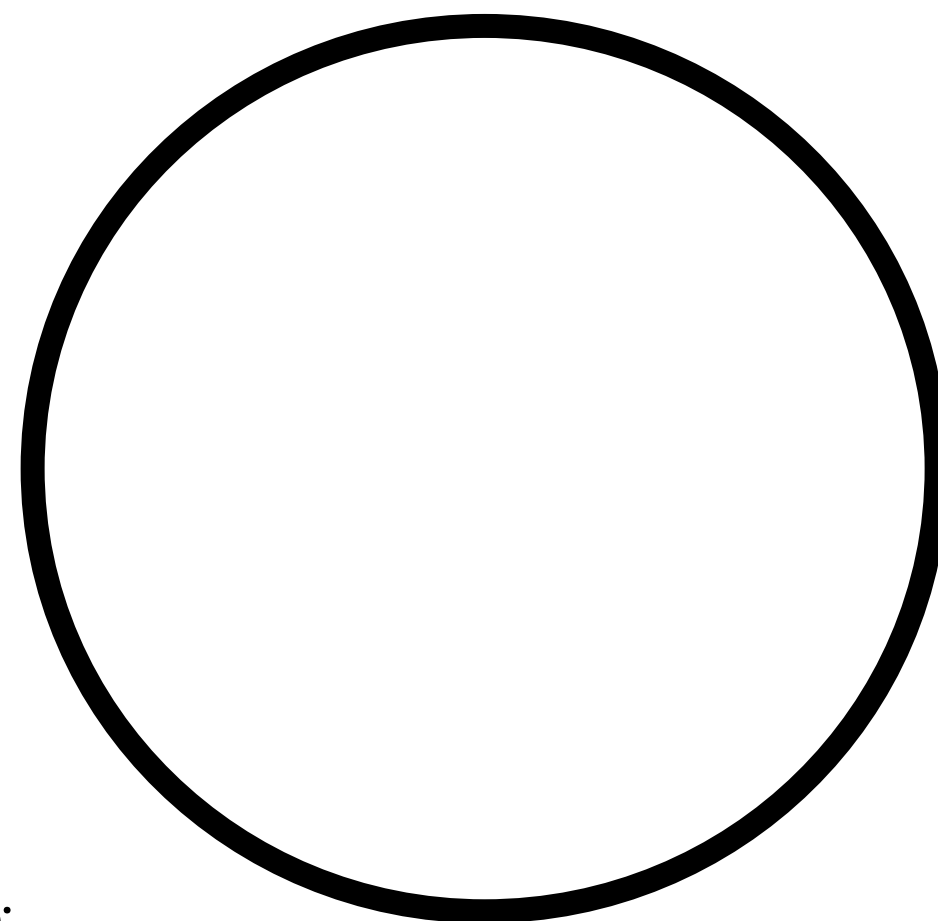
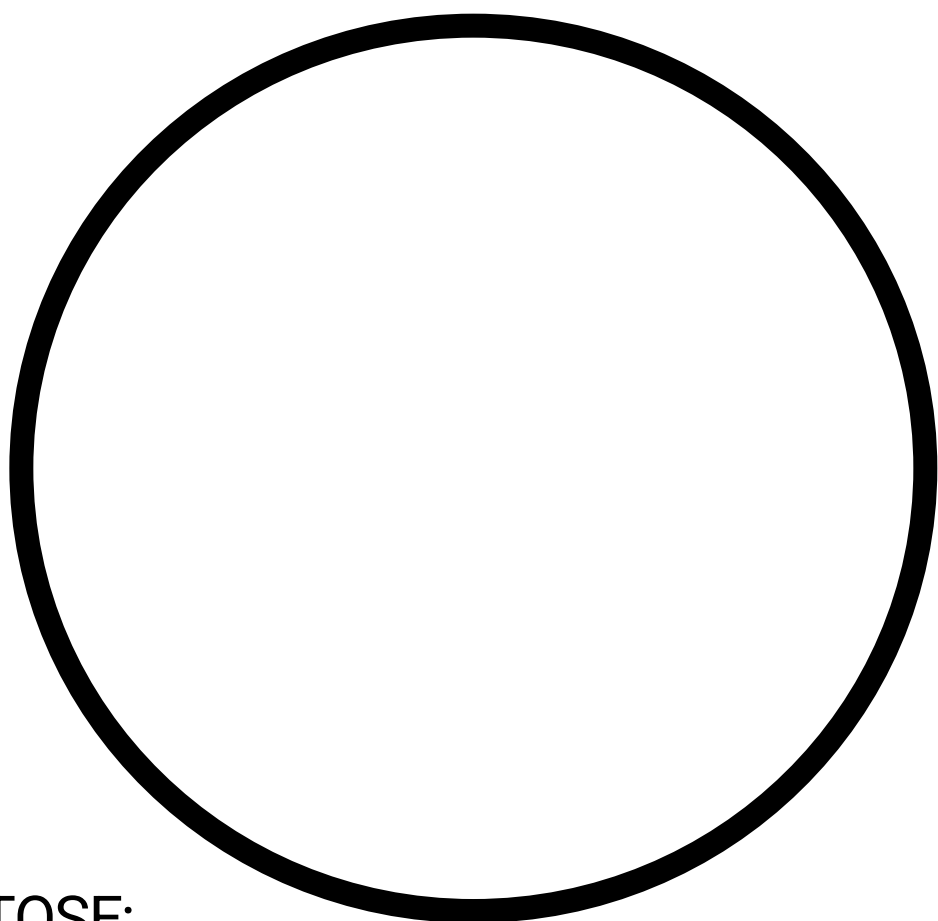


GLUCOSE: _____

Reference:

Actual Specimen

Researched Specimen

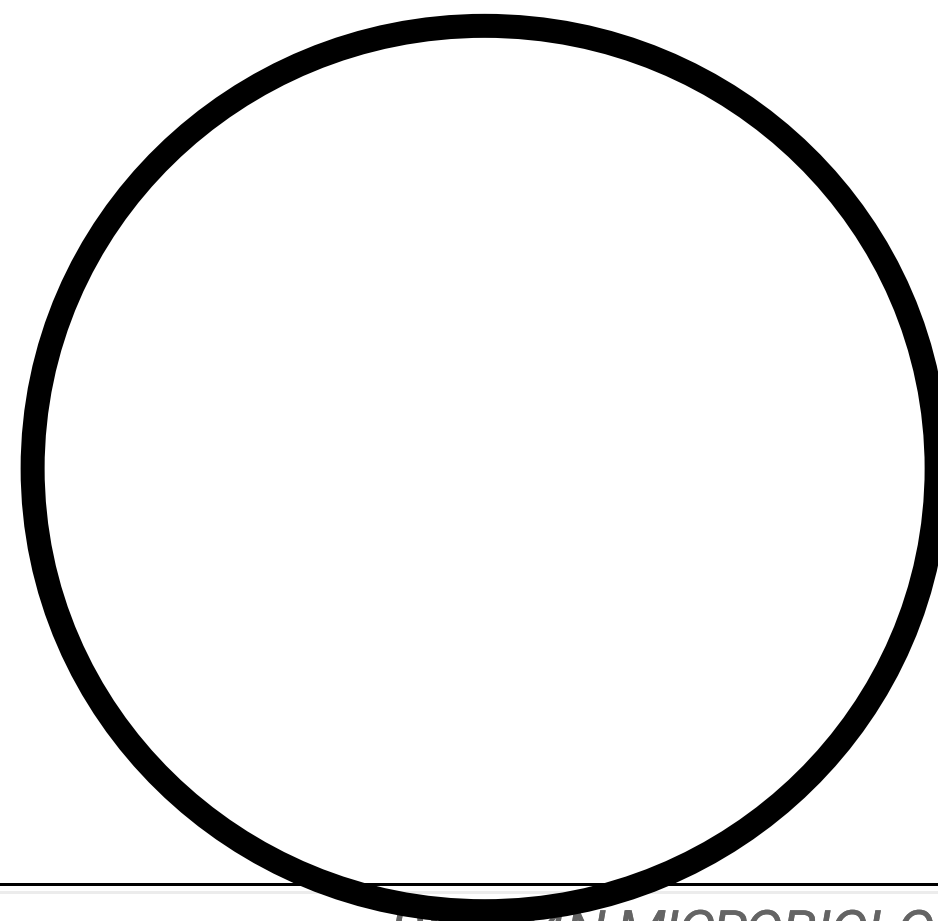
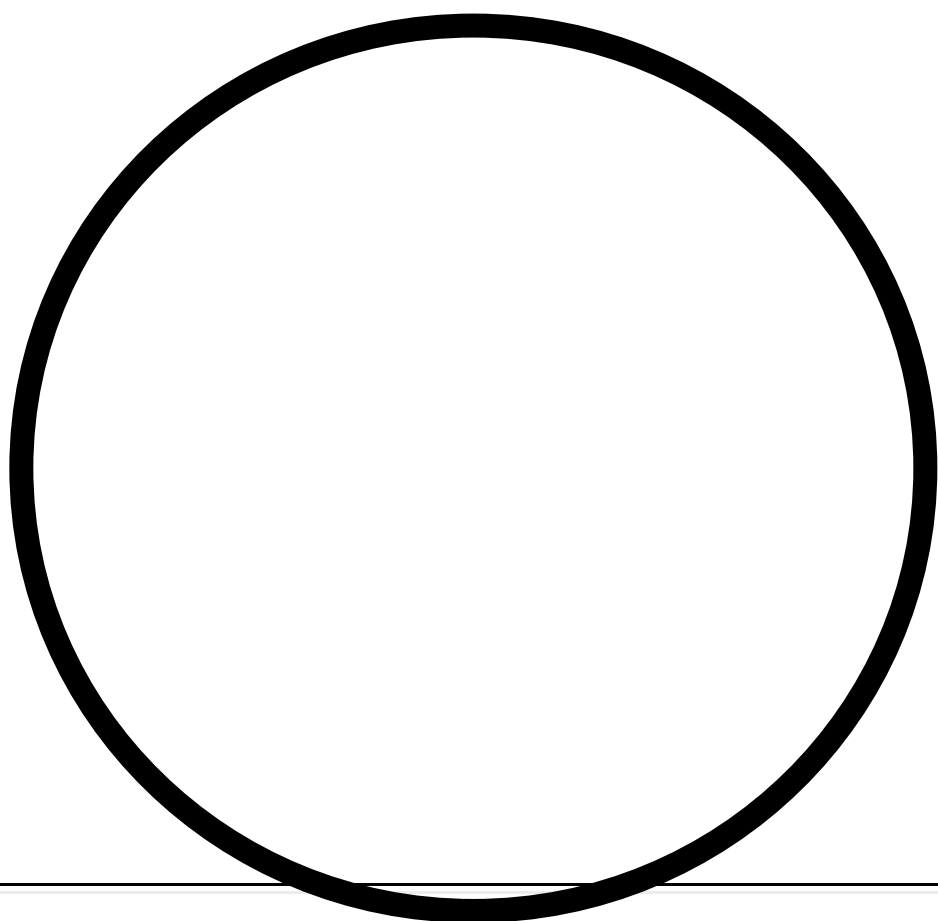


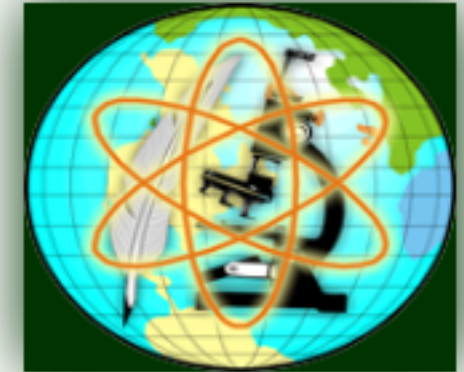
LACTOSE: _____

Reference:

Actual Specimen

Researched Specimen





SUCROSE: _____

Reference:

Record the results in the table below.

The following convention is used when noting the results of fermentation experiments.

A = acid

G = gas

AG means that both acid and gas are present

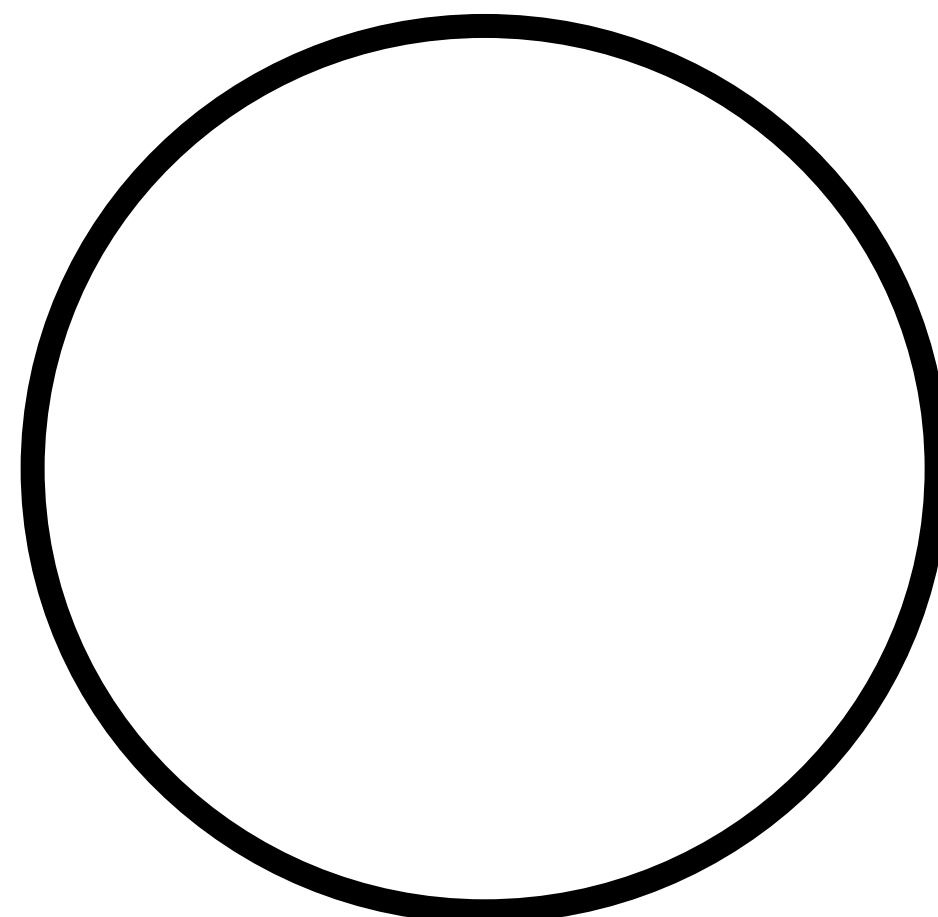
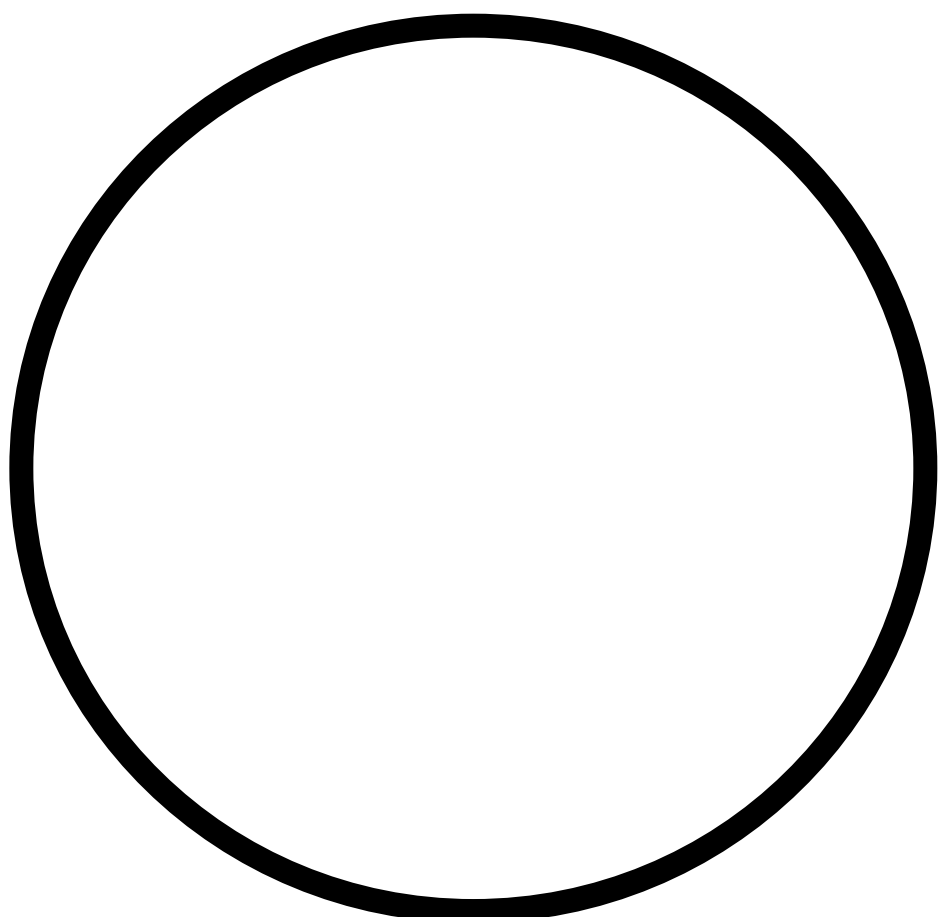
If neither acid nor gas is present, you can write "negative"

BACTERIA	GLUCOSE	LACTOSE	SUCROSE
<i>Proteus vulgaris</i>			
<i>Escherichia coli</i>			
<i>Streptococcus aureus</i>			

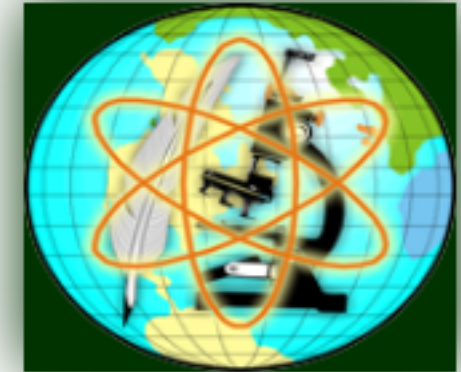
B. CASEIN HYDROLYSIS

Actual Specimen

Researched Specimen

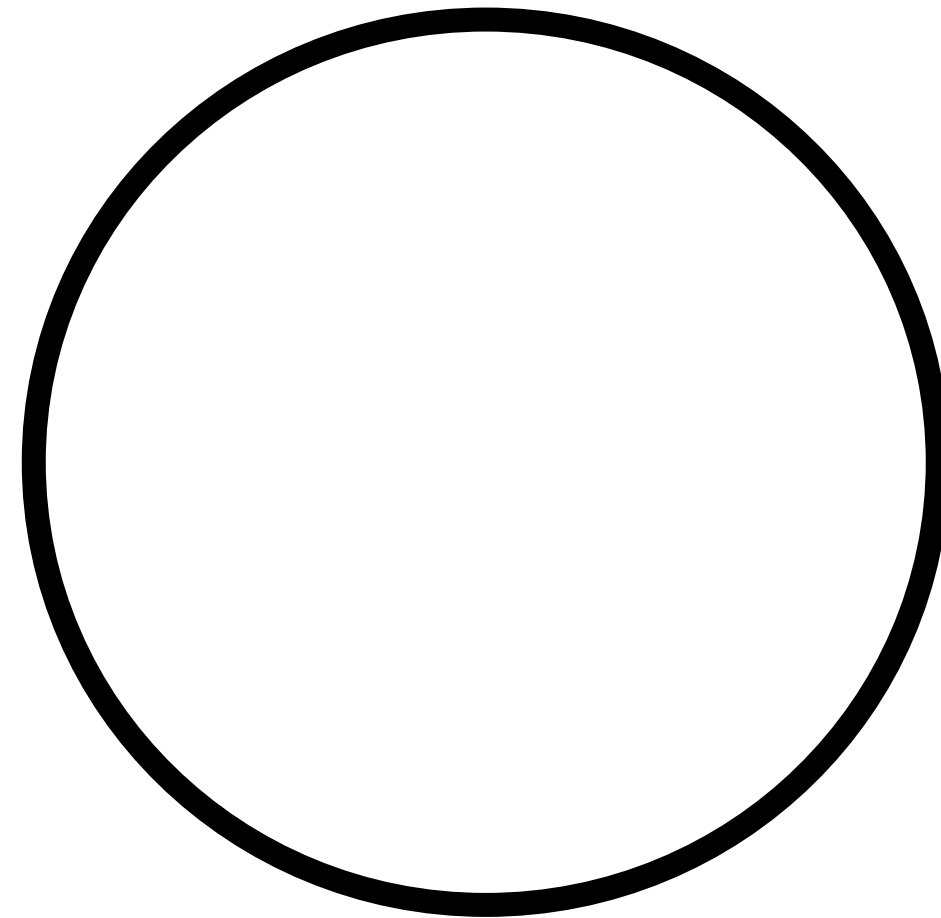
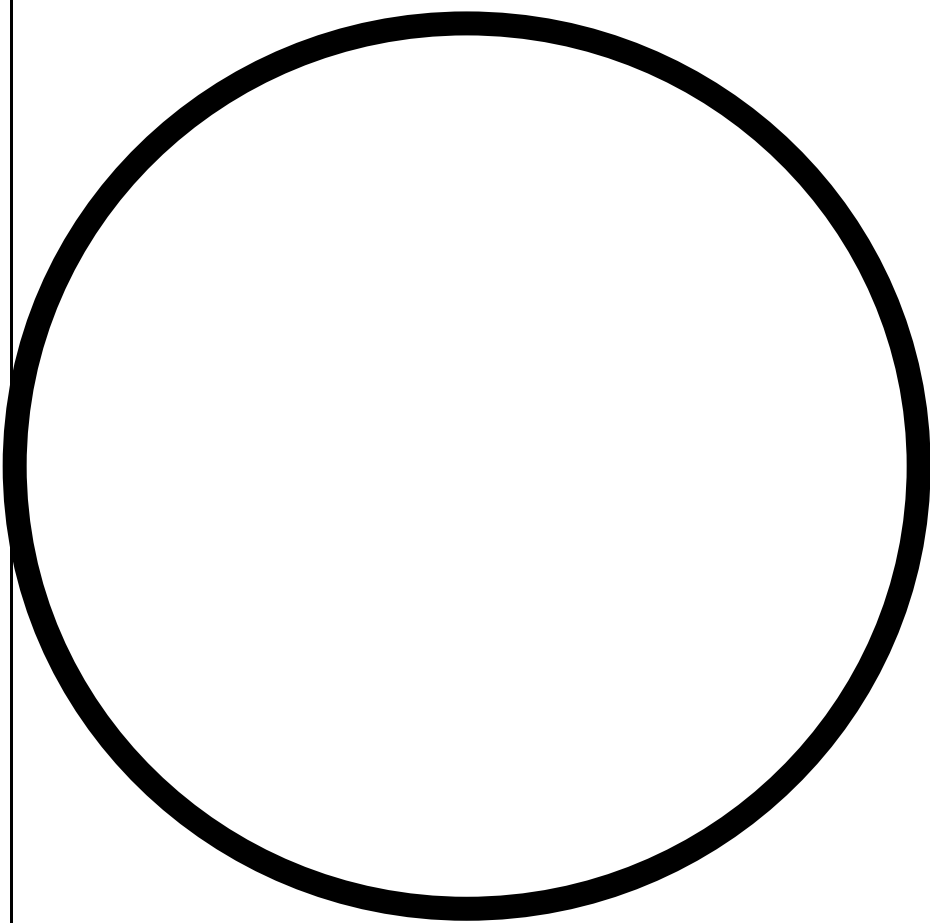


BACTERIA	CLEAR ZONE?	RESULT (+/-)
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<i>Proteus vulgaris</i>		
<i>Escherichia coli</i>		
<i>Streptococcus aureus</i>		

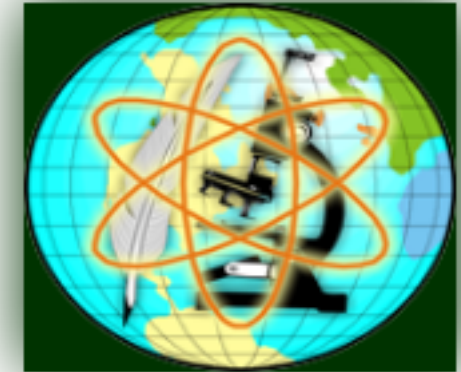
C. CITRATE UTILIZATION



BACTERIA	COLOR	RESULT (+/-)
<i>Proteus vulgaris</i>		
<i>Escherichia coli</i>		
<i>Streptococcus aureus</i>		

TEST	Biochemical reaction/ Enzyme involved	Medium used/ Inoculation Procedure	Appearance of Result		What causes the change in the medium's appearance? (e.g. pH change, enzyme activity, etc.)
			+	-	
Carbohydrate Fermentation					
Casein Hydrolysis					
Citrate Utilization					

V. GENERALIZATION



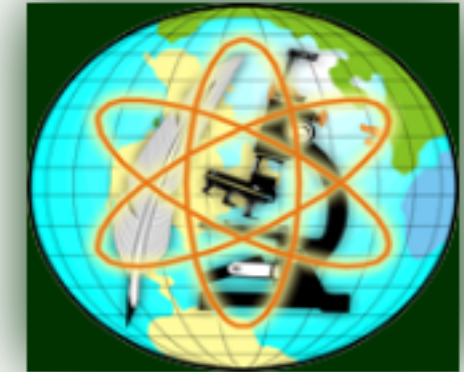
VI. REFERENCES

VII. QUESTIONS FOR RESEARCH (Cite references right after your answers) (30 points)

1. Distinguish between respiration and fermentation (5 points).
2. Do all microorganisms use pyruvic acid in the same way? Explain (5 points)
3. Describe a pathway used for the degradation of carbohydrates by strict anaerobes (5 points).
4. Simmons citrate medium contains primarily inorganic ammonium, potassium, and sodium salts, plus organic



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citrate. What is the rationale for using a medium with this type of composition for the performance of the citrate utilization test?

5. Account for the development of alkalinity in cultures capable of using citrate as their sole carbon source.

**NOTE: May use extra sheets of paper for your answers.*

Let be guided by these rubrics.

RUBRICS FOR ESSAY

Score	Completion	Accuracy	Comprehension	Organization	Conventions
5	The answer is complete.	All information provided is accurate.	Content demonstrates a deep understanding of, and engagement with, the text(s).	Content is well-organized and easy to read. Points follow a logical progression.	No major grammatical or spelling errors. No more than two minor errors.
4	The answer is missing slight details	All information provided is accurate.	Content demonstrates understanding of, and engagement with, the text(s).	Content is well-organized and easy to read. Points follow a logical progression.	No major grammatical or spelling errors. No more than five minor errors.
3	The answer is missing multiple details.	Most information provided is accurate.	Content demonstrates basic understanding of the text(s).	Content is organized and easy to read. Points follow a mostly logical progression.	Some major and minor errors that don't necessarily impair communication.
2	Content suggests lack of preparation or comprehension.	Some information provided is accurate.	Content demonstrates less than basic understanding of the text(s).	Content may be unorganized and difficult to read. Points do not follow a solidly logical progression.	Major and minor errors significantly weaken quality of communication, although still comprehensible.
1	Content only marginally related to the question/prompt.	A small amount of the information is accurate.	Content demonstrates a lack of understanding of the text(s).	Content is unorganized, illogical, and difficult to read.	Communication seriously impaired by multitude of spelling/grammatical errors.
0	Content fails to meet the basic requirements of the task.	None of the information provided is accurate.	Content demonstrates a complete lack of understanding of the text(s).	Content is very poorly organized, illogical, and difficult to read.	Multitude of major and minor errors make answer incomprehensible.