PRACTICAL PUPIL WORK WITH YEASTS AND BACTERIA.¹

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I have an impression, whether right or wrong, that a good many biology teachers refrain from attempting much practical pupil work with yeasts and bacteria because they feel that such work requires much time in preparation and an extensive and expensive laboratory equipment. The preparation of good exercises with these organisms does require more time than that of many other laboratory studies, I will admit, but the idea that an elaborate equipment is necessary is not correct. Some of the best exercises can be performed with the simplest materials.

At the risk of stirring up the old question as to which shall precede, structure or function, I shall suggest beginning the work on yeast with the microscopic study. The best medium I have found for growing yeast for microscopic work is Pasteur's Solution, and the most favorable temperature about 28° C. In this fluid and at this temperature the yeast plants develop rapidly, and within a day or two one can be sure of an abundance of chains and buds. Pasteur's Solution can be easily prepared according to the following formula:

H₂O, 850 g.; (NH₄)₂C₄H₄O₆, 10 g.; K₃PO₄, 2 g.; Ca₃(PO₄)₂, .2 g.; MgSO₄, .2 g. For fermentation as well as growth, add C₆H₁₂O₆, 150 g.

This will give enough material to supply two or three classes for two years. The solution should be kept in a glass stoppered bottle and sterilized each time the bottle is opened; otherwise it will be quickly spoiled by the yeasts and molds which will develop in it.

An exercise on fermentation may follow the microscopic study. The yeast and sugar solution may be placed in Erlenmeyer flasks fitted with delivery tubes, and the CO₂ collected in test tubes or cylinders loosely plugged with cotton. The pupils can note the difference in sweetness in the solution before and after the action of the yeast, and make their own CO₂ tests. I have not found it practical to have the pupils make individual tests for alcohol; instead they pour the contents of their flasks into one large round-bottomed flask, and we distill off enough of the alcohol to detect its odor. Perhaps some of you can suggest some better way of demonstrating the disappearance of the sugar. I have not found the Fehling's test satisfactory, as so much of the sugar remains unaltered at the end of the experiment. The practical application of this experiment needs no comment.

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A third experiment with yeast, and one of which I am particularly fond, has four distinct objects: To determine whether growth can take place without fermentation; to determine whether fermentation can take place without growth; to determine the elements or compounds necessary for growth; to determine the elements or compounds necessary for fermentation. This experiment requires no apparatus except fermentation tubes, yeast water, the various culture media employed, and a warm place in which to keep the tubes. The following table shows the method of recording results:

Table A.
Data.

Contents of tubes.	Elements or compounds.	Volume of gas.	Turbidity.	Fermentation.	Growth.
1 Water.....	H ₂ O	0 c. c.	None	No	No
2 Water.....	H ₂ O	0 c. c.	None	No	No
3 Sugar.....	C ₆ H ₁₂ O ₆	3 c. c.	Trace	Yes	No
4 Sugar.....	C ₆ H ₁₂ O ₆	5 c. c.	Trace	Yes	No
5 Beef juice.....	C, H, O, N, S, Ca, Mg, K	0 c. c.	Much	No	Yes
6 Beef juice.....	C, H, O, N, S, Ca, Mg, K	0 c. c.	Much	No	Yes
7 Beef juice and sugar.....	C, H, O, N, S, Ca, Mg, K + C ₆ H ₁₂ O ₆	6.4 c. c.	Much	Yes	Yes
8 Beef juice and sugar.....	C, H, O, N, S, Ca, Mg, K + C ₆ H ₁₂ O ₆	6.7 c. c.	Much	Yes	Yes
9 Pasteur's solution.....	S, Ca, Mg, K H, O, N, C, P, Ca, Mg, K	0 c. c.	Much	No	Yes
10 Pasteur's solution.....	H, O, N, C, P Ca, Mg, K	0 c. c.	Much	No	Yes
11 Sugar and Pasteur.....	H, O, N, C, P Ca, Mg, K + C ₆ H ₁₂ O ₆	9.5 c. c.	Much	Yes	Yes
12 Sugar and Pasteur's solution.....	H, O, N, C, P, Ca, Mg, K, + C ₆ H ₁₂ O ₆	9.2 c. c.	Much	Yes	Yes

I have found that the success of this experiment depends almost entirely upon the method of filling the fermentation

tubes. Two drops of yeast water should be placed with a pipette or ink-dropper in the bend of the tube, then the long arm filled with the culture medium. This fluid should just seal the bend, but not extend up into the bowl.

Almost invariably the pupils approach this experiment with the preconceived idea that fermentation and growth are inseparable. It gives them a little lesson in drawing their conclusions from facts rather than from opinions.

There are many experiments which can be performed with bacteria. I will mention only five: First, the microscopic study. The microscopic study of living bacteria is useful in giving the pupils a conception of the intense activity of these organisms. One glance into a drop of beef broth or hay infusion full of wiggling bacilli and twisting spirilla is worth a dozen sermons on hygiene. High powered microscopes are not necessary for this work; a magnification of 500 diameters will show many forms. A saucer of beef broth or hay infusion left exposed to the outdoor air for several hours then kept in a flask in a warm place for two days will furnish an abundance of material. To study carefully the forms of different bacteria stained slides and charts or illustrations are necessary. The teacher can prepare slides without great difficulty or, if she prefers, she can doubtless borrow them from the local board of health. The M. J. Breitenbach Co., of N. Y., manufacturers of Peptomangan, publish an excellent little chart which they will send to a teacher free of charge.

The second experiment has for its object the study of the effects of single and discontinuous sterilization on bacteria. The pupils fill with beef broth and peptone, furnished by the instructor, 12 test tubes for each laboratory table. These they divide into three lots. The first lot they sterilize 135 minutes; the second and third 45 minutes each. The next day they sterilize lots two and three 45 minutes; the third day, lot three 45 minutes. Between and after sterilization the tubes are kept warm. Two or three days after the last sterilization all the tubes are ready for examination. Results may be recorded as in table B on page 798.

The application of this exercise to vegetable canning this summer is evident. Without doubt the food could be sterilized by a single sterilization continued for several hours, but the shorter sterilizings on three consecutive days would certainly consume much less gas.

For the third experiment the pupils are furnished with sterile agar tubes. These they inoculate from the mucous membrane of the mouth, from under the finger-nail, from the skin of an apple purchased at an out-of-door fruit stand, from a dollar bill, etc. After the tubes have incubated two days, they examine them and observe the colonies of bacteria that have developed. There are always a few pupils who are sufficiently interested in the work to make microscopic examinations of the colonies.

Table B.
Data.

Table No.	Sterilized once 135 min.		Sterilized twice 45 min. each time		Sterilized 3 times 45 min. each time	
	Bacteria		Bacteria		Bacteria	
	Present	Absent	Present	Absent	Present	Absent
1.	1	3	1	3	0	4
2.	1	3	1	3	0	4
3.	1	3	0	4	0	4
4.	1	3	1	3	0	4
5.	2	2	0	4	0	4
6.	1	3	0	4	0	4
Totals	7	17	3	21	0	24

I have observed that this experiment appeals quite strongly to the sporting instincts of the boys, for frequently I notice them making wagers—after class, of course—as to which one will have the smallest number of colonies develop from his mouth. A pupil who obtains a flourishing colony of catarrh germs from under the finger-nail, as one of mine did this year, will probably be more careful to keep the fingers out of the mouth and nose henceforth.

In the ventilating system of the Plainfield High School, the heated air is forced through a chamber in which a spray of water is falling. This is supposed to wash the bacteria and dust out of the air before it reaches the classrooms. Each year we test the efficiency of this apparatus by exposing twelve sterile agar plates in petrie dishes for fifteen minutes in twelve different rooms, and at the same time twelve more plates at different points around the outside of the building. After the plates have incubated two days, the pupils examine them and compare the number of colonies on the plates exposed indoors with those on the plates exposed outside. They record

the results in tabular form, calculating the average number of colonies indoors and outdoors. The following chart indicates the result of our experiment this winter, and this is by no means an unusual result:

Table C.
Data.

	Location in building Room No.	Number of colonies		Location outside of building	Number of colonies
1	120	8	1	East	35
2	331	3	2	West	10
3	Library	0	3	Front	100
4	160	0	4	Back	25
5	318	1	5	West	25
6	270	3	6	East	9
7	261	0	7	West	18
8	340	5	8	Back	3
9	351	1	9	Front	12
10	338	3	10	East	200
11	221	0	11	East	50
12	101	8	12	West	9
Total No.....		32	Total No.....		496
Average		3	Average		41

It certainly demonstrates to the pupils and their parents the efficiency of our ventilation system in removing bacteria from the air. Perhaps it is only fair to the Plainfield atmosphere to state, however, that I always perform this experiment if possible on a windy day when plenty of germs are being blown around outside. The results are much more startling.

The object of the last experiment is to determine the effects of pasteurizing and sterilizing on milk. The pupils fill 216 tubes with raw milk. These they divide into three lots; the first lot they leave raw, the second they pasteurize, the third they sterilize. Each lot they then divide into two sets, one of which they place in a refrigerator, the other in the incubator or some other warm place. The next day they examine one-third, the following day another third, and the next day the remainder. This table shows the form of recording observations.

It shows them, among other things, that pasteurized milk will not keep indefinitely, as many people think. It indicates also the action of lactic bacteria in checking the growth of putrifying bacteria in the human intestine. It does not indicate anything, however, as to the effects of pasteurizing and sterilizing on the digestibility of milk.

Table D.
Data.
Condition of Milk.

Time	Raw		Pasteurized		Sterilized	
	Cold	Warm				
1 day.....	12 normal	12 sour	12 normal	11 normal 1 sour	12 normal	12 normal
2 days....	12 normal	12 sour	12 normal	6 putrid 5 normal 1 sour	12 normal	12 normal
3 days....	7 sour 5 normal	12 sour	12 normal	12 putrid	12 normal	12 normal

I have said that the apparatus required for these experiments is neither elaborate nor expensive. A microscope with an oil-immersion lens is a help but not a necessity. A good incubator can be procured from Eimer & Amend for about \$25, but this is also not a necessity. Before I had one, I used to place my cultures on top of the boilers in the engine room. If you cannot afford an Arnold sterilizer, you can substitute a large double boiler. For pasteurizing, a tin kettle with a hole punched through the cover for the insertion of a thermometer will do very well.

The glassware and other apparatus required consists of twelve small fermentation tubes, one or two gross of heavy test tubes without lip, 5 x 5/8 inches (these can be obtained from the Williamstown Glass Co., Williamstown, N. J., for \$2.20 per gross), a few wire baskets to hold the test tubes, pipettes or ink-droppers, inoculation needles, twenty-four petrie dishes, one or more wax pencils for labeling the tubes and petrie dishes, and a supply of absorbent cotton for plugs. Glass tubing, flasks, etc., can be borrowed from the chemical laboratory if necessary. Stains can be prepared easily by the teacher or purchased at small cost. The local board of health will probably be glad to supply agar shreds and peptone or prepared agar in small quantities at a nominal price, and to give suggestions as to the preparation of materials.

A supply of glass cleaning mixture is necessary for cleaning fermentation tubes, and saves much time and energy in cleaning test tubes and petrie dishes. To prepare it, dissolve 80 g. $K_2Cr_2O_7$ in 300 c. c. hot H_2O and add to this drop by drop from a burette 460 c. c. H_2SO_4 . This solution will remove organic matter from glassware in from fifteen minutes to an hour. It can be used repeatedly till the red crystals disappear and the mixture begins to turn green.

The experiments I have outlined are valuable, it seems to me, because they give the pupils a little insight into bacteriological methods, and substitute a definite conception of the forms and activities of yeasts and bacteria for the more or less hazy idea that most people have of "germs." They have practical applications to the pupils' daily lives and personal hygiene. They are more stimulating than many of the available experiments in plant physiology, in that their results cannot be foretold by the pupil. If a student has even a mediocre imagination, or has used his eyes to any advantage, he can make a pretty shrewd guess as to what will happen when the cotyledons are removed from a seedling, when a stem is placed in a bottle of red ink, or when a plant is grown in a box illuminated from one side only. He cannot tell what will happen when yeasts and bacteria are grown under different conditions, hence a greater interest in the experiment.

But the greatest value of a number of these exercises lies, I think, in the fact that their observations can be recorded, as in many physics experiments, in tabular form, giving the pupils training not only in accurate observations but in thinking out logical conclusions from their data. Such experiments have a distinct advantage, I believe, over many of the dissections and living studies carried on either in the laboratory or the field, which train the pupils in observation and in ability to describe what they see but not in reasoning power.

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