**Enzyme Preps**

 **Beano** as source of α-galactosidase enzyme (exogenous source)

1. Add 3 Beano tablets to mortar
2. Grind to fine powder using pestle
3. Transfer powder to 100 ml beaker labeled “Beano”
4. Add 6-8 drops of distilled water to powder and stir to form a smooth paste
5. Add distilled water to beaker with paste up to 50 ml mark on beaker
6. Mix carefully
7. Store on ice until needed

 **Lactaid** as source of β-galactosidase enzyme (exogenous source)

1. Add 2 Lactaid tablets to mortar
2. Grind to fine powder using pestle
3. Transfer powder to 100 ml beaker labeled “Beano”
4. Add 6-8 drops of distilled water to powder and stir to form a smooth paste
5. Add distilled water to beaker with paste up to 50 ml mark on beaker
6. Mix carefully
7. Store on ice until needed

 **Water** – distilled water as control (no additional exogenous enzyme)

**Fermentation Tubes:**

1. Label fermentation tubes and sort into 3 groups on the basis of enzyme prep to be added
2. Add **10 ml** of the yeast suspension to all the tubes except tubes 5 and 10 which should get 10 ml of water in place of the yeast suspension
3. Add **2 ml** of the appropriate enzyme preparation or the distilled water all of the 16 tubes.
	1. Tubes 1-6 each get 2 ml of the Beano prep
	2. Tubes 7-12 each get 2 ml the Lactaid prep
	3. Tubes 15 and 16 get 2ml of the distilled water
4. The last addition to each tube will be **4 ml** of the different sugar substrates or the distilled water
	1. Galactose into tubes 1, 7 and 13
	2. Glucose into tubes 2, 8, and 14
	3. Lactose into tubes 3, 9, 10 and 15
	4. Melibiose into tubes 4, 5, 11 and 16
	5. Water into tubes 6 and 12
	6. Cover the open end of the tube carefully with parafilm.
5. After the last sugar/water control is added start timer for 25 minute incubation period
	1. Read level of gas displacement in the fermentation tubes in terms of ml of displacement in the closed vertical scored rounded top of the tube.



Read volume displaced by gas in ml

Cover with parafilm

Experimental Set Up: note change from course handout (I added a few additional controls if we have enough fermentation tubes available and I organized the tubes on the basis of the enzyme prep to be added.)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tube** | **Substrate (Sugar)** | **Enzyme Prep** | **Yeast** | **Gas (CO2****)** **Displacement** |
|  | 4 ml | 2 ml | 10 ml  | ml  |
| 1\* | Galactose | Beano | Added |  |
| 2\* | Glucose | Beano | Added |  |
| 3 | Lactose | Beano | Added |  |
| 4 | Melibiose | Beano | Added |  |
| 5\* | Melibiose | Beano | Water |  |
| 6 | None  | Beano | Added |  |
| 7 | Galactose | Lactaid | Added |  |
| 8 | Glucose | Lactaid | Added |  |
| 9 | Lactose | Lactaid | Added |  |
| 10\* | Lactose | Lactaid | Water |  |
| 11 | Melibiose | Lactaid | Added |  |
| 12 | None  | Lactaid | Added |  |
| 13 | Galactose | Water | Added |  |
| 14 | Glucose | Water | Added |  |
| 15 | Lactose | Water | Added |  |
| 16 | Melibiose | Water | Added |  |