Unit 15 application of biotechnology: assignment 3: enzyme technology

Technology

Task 1: Introduction In this task I will investigate how the enzyme lactase breaks down the disaccharide lactose into monosaccharides, which are glucose and galactose. The main aim will be to get lactose – free milk as milk contains lactose. When we drink milk we need lactase (enzyme) to break down the lactose, which is contained by the milk. Once I have created my own method of enzyme immobilisation of lactose using lactase I will then construct a bioreactor. I have looked at and researched on the Internet and found many ways (methods) to carry out this experiment.

I have also noticed that some are long and complicated to understand and follow but I have managed to get one decent method as a basis for my own method to be created. These have been attached at the appendix. Plan I will carry out this experiment using 5 different temperature ranges, as this will give me good, accurate and reliable results. It will give me more data of results to compare and analyse. So the 5 temperature ranges that I have chosen are: 30°C, 35°C, 40°C, 45°C and 50°C (independent variables). I will also repeat the experiment 3 times for each temperature to get more reliable results.

I will make sure the time the mixtures are kept in the water baths are temperature equilibrium, which means that they will be kept in the water baths until they reach that temperature. This will require me to use a thermometer and place it in with the mixtures (5mins or more). Apparatus: •2cm3 lactase enzyme •8cm3 Sodium alginate solution •100cm3 Calcium chloride solution •Hot water baths set at 30°C, 35°C, 40°C, 45°C and 50°C. •Small beakers x 4 •Syringes 5cm3 (precision +/- 0. 05cm3) •Glucose strips/reagent strips Semi-quantitative (Diabur 5000)(max 3%) •100cm3

beaker Test tubes and rack •Glass rod •Plastic tea strainer •Distilled water 100cm3 •Pasteurised milk (semi-skimmed) •Stop watch (+/- 0. 05sec) •Thermometer Method 1. First get all the apparatus out and set up. 2. Mix 8cm3 sodium alginate solution with 2cm3 lactase enzyme solution in a beaker, and transfer into a syringe. 3. Add this mixture drop-wise into the calcium chloride solution (100cm3). Alginate beads will form in the beaker, leave for 15 to 20 mins for the beads to harden. 4. Strain off the beads using the tea strainer and rinse with distilled water. . Transfer the beads into a test tube. 6. Place the test tube containing the beads and a test tube containing 2cm3 of milk into a water bath of 30°C and wait until they have reached the temperature of the water bath. (About 5mins). 7. Then pour the milk in the test tube into the test tube with the beads and start the stopwatch immediately. 8. After 5mins test the solution with a glucose strip and record the amount of glucose present. 9. Repeat this for each temperature 3 times. 10. Repeat this for 35°C, 40°C, 45°C and 50°C.

Note: the alginate beads can be re-used after been rinsed with distilled water. Then this experiment method can also be used for the mobilised free enzyme. This can be done by simply repeating the above method but without adding calcium chloride which means that steps 1, 2 and 3 don’t need to be taken. Reference: Michael Roberts 2001 http://www. 123helpme. com/view. asp? id= 148785 A simple batch bioreactor to be used by both immobilised and mobilised enzyme (lactase) Building a coke Bottle Bioreactor Purpose: Coke bottle bioreactors are designed to be used as tools for composting research.

They are small and inexpensive enough to enable students to design and carry out individualized research projects, comparing variables such as reactor design, moisture content, and nutrient ratios of mixtures to be composted. This is simply a batch bioreactor, which means that it is not circulated. Materials: •Two 2-litre or 3-litre coke bottles •One smaller container, about 5-cm high, that fits inside the soda bottle •One Styrofoam plate or tray •Drill or nail for making holes •Duct tape or clear packaging tape •Utility knife Insulation materials such as sheets of fibreglass or foam rubber, or Styrofoam peanuts •Fine-meshed screen or fabric large enough to cover top of soda bottle and air holes in bottom half •Thermometer that will fit into the top of the soda bottle and be long enough to reach down into the centre of the compost •Chopped vegetable scraps such as lettuce leaves, carrot or potato peelings, and apple cores, or garden wastes such as weeds or grass clippings •Bulking agent such as wood shavings or 1-2 cm pieces of paper egg cartons, cardboard, or wood •Optional: hollow tubing to provide ventilation

Construction: Using a utility knife or sharp-pointed scissors, cut the top off one coke bottle just below the shoulder and the other just above the shoulder. Using the larger pieces of the two bottles, you will now have a top from one that fits snugly over the bottom from the other. Place a smaller container (roughly 4-5 cm high) upside down into the bottom of the coke bottle. This will form a stand to support the tray that will hold the compost. You can use any plastic container that will fit inside the bottle and provide adequate support for the Styrofoam stand and overlying compost.

The next step is to make a Styrofoam circle. Trace a circle the diameter of the soda bottle on a Styrofoam plate or try and cut it out, forming a piece that fits snugly inside the soda bottle. Use a nail to punch holes through the Styrofoam for aeration. Assemble the bottom of your bioreactor by placing the stand into the coke bottle, then resting the Styrofoam circle on top of the stand. Make a mark on your bottle to indicate where the Styrofoam circle sits. Above this point is where the compost will be, and below it is where you want to make air holes.

Make air holes in the sides of the coke bottle in the area below the mark that you made. This can be done with a drill or by carefully heating a nail and using it to melt holes through the plastic. Avoid making holes in the very bottom of the bottle unless you plan to use a tray underneath to collect whatever leach ate may be generated during composting. Reassemble the bioreactor pieces, making sure that you have provided sufficient air holes to allow air to enter the bottle and flow up through the stand and Styrofoam circle. Fill the bioreactor with the mixture you wish to compost.

A variety of materials will work, but in general you want a bulking agent to provide airflow plus some vegetable scraps to provide food for the microbes (see following table for some possibilities). Reference: http://www. css. cornell. edu/compost/soda. html Factors that need to be kept constant During the experiment there are few factors that affect the outcome of the experiment and these need to be kept constant. These are: making sure that the Ph doesn’t change, the temperature needs to be constant and there is plenty of oxygen as mentioned earlier on via bioreactor.

The lecture then decided to conduct the experiment as a class and this meant that there were a few changes made but the basis of the experiment still remained. These changes were the temperatures that were changed to 25°C, 30°C, 35°C, 38°C, 45°C and 50°C. Another change was the concentration of the sodium alginate, which was changed to 1. 5%. The other change that was made to the method above due to carrying out experiment as a class was the bioreactor. Instead of making a bottle bioreactor it was decided upon the use of a large beaker as a bioreactor.

Another change was made that was the reagent strips to test for glucose levels and these were changed from the original ones that were decided to be used at first to clinistix - diastix. These were dipped for a certain time and then let dry for 30 seconds before taking the reading by matching the colour on the strip by the colours on the small bottle. Another change was made affecting the time spent on the experiment and this was the beads were left well 5 minutes to harden instead of leaving them for 10 – 15mins.

The last change made to the method was that the there were going to be 2 readings taken one reading after 5mins and the other after 10mins and these were repeated. So the final method and apparatus that were actually used were: Apparatus •2cm3 lactase enzyme •8cm3 Sodium alginate solution •100cm3 Calcium chloride solution •Hot water baths set at 30°C, 35°C, 40°C, 45°C and 50°C. •Small beakers x 4 •Syringes 5cm3 (precision +/- 0. 05cm3) •Glucose strips/ diastix reagent strips Semi-quantitative •100cm3 beaker •Test tubes and rack •Glass rod •Plastic tea strainer •Distilled water 100cm3 Pasteurised milk (semi-skimmed) •Stop watch (+/-

0. 05sec) Method (mobilised enzyme) 1. First get all the apparatus out and set up. 2. Mix 8cm3 sodium alginate solution with 2cm3 lactase enzyme solution in a beaker, and transfer into a syringe. 3. Transfer this in to a test tube from the syringe. 4. Pour in 2cm3 of milk in a test tube. 5. Place the two test tubes in a water bath of 25°C and wait until they have reached the temperature of the water bath. (About good 5mins). 6. Then pour the milk in the test tube into the test tube with the lactase free enzyme solution and start the stopwatch immediately. . After 5mins test the solution with a diastix reagent strip and record the amount of glucose present. 8. Then After 10mins test the solution with a diastix reagent strip and record the amount of glucose present. 9. Repeat this again for the same temperature. 10. Repeat this for 30°C, 35°C, 38°C, 45°C and 50°C. Method (immobilised enzyme) 1. First get all the apparatus out and set up. 2. Mix 8cm3 sodium alginate solution with 2cm3 lactase enzyme solution in a beaker, and transfer into a syringe. 3. Add this mixture drop-wise into the calcium chloride solution (100cm3).

Alginate beads will form in the beaker, leave for 5mins. 4. Strain off the beads using the tea strainer and rinse with distilled water. 5. Transfer the beads into a test tube. 6. Pour in 2cm3 of milk in a test tube. 7. Place the test tube containing the beads and a test tube containing 2cm3 of milk into a water bath of 25°C and wait until they have reached the temperature of the water bath. (About good 5mins). 8. Then pour the milk in the test tube into the test tube with the beads and start the stopwatch immediately. 9.

After 5mins test the solution with a diastix reagent strip and record the amount of glucose present. 10. Then After 10mins test the solution with a diastix reagent strip and record the amount of glucose present. 11. Repeat this again for the same temperature. 12. Repeat this for 30°C, 35°C, 38°C, 45°C and 50°C. As you can see in the previous pages there are results of both free enzyme and immobilised enzyme experiments of the whole class. As you can also see that there were two readings taken of two different times (also mentioned in the final method).

But were advised to analyse only one reading and it was decided that the readings gained from the 5 minutes experiment were the appropriate ones. Calculating the rates of results To calculate the rate of something you do the following: For example: For the temperature 25°C of the immobilised lactase enzyme at 5mins there were readings of: 2. 5, 5, 2. 5, 2. 5, 2. 5, 2. 5, 2. 5, 2. 5, 2. 5, 5, 5, 5 and these will have to be added up and the total = 40 which you divide by 512 because this was the amount of numbers that were totalled up. 0 = 3. 33g/l/5mins 12 You now divide this number by 5 giving you grams per litre per a minute. 3. 33 = 0. 66g/l/a minute 5 This calculation whole will now be conducted with the rest of the results including the mobilised enzyme. Below is a table containing the rates of both immobilised and mobilised lactase of 5 minutes. Results of immobilised & mobilised lactase of rates: Temperature (°C)Immobilised enzymeMobilised enzyme 250. 661. 06 301. 751. 75 353. 662. 75 384. 002. 38 452. 541. 25 501. 000. 1

The results above in the table are shown in a line graph and in a barchart to get a better understanding of the comparison that is going to be about the differences between the immobilised and mobilised lactase enzyme. In the above graphs you can see that the glucose levels at the 25°C are higher for the mobilised enzyme (1. 06g/l per minute) than the immobilised lactase (0. 66g/l per minute. Then in the 30°C temperature the levels for both lactase enzymes increase to the same levels of 1. 75g/l per minute. Then in the 35°C the levels of both rise again but this time the immobilised lactase enzyme (3. 6g/l per minute) increases more than the mobilised lactase (2. 75g/l per minute). In the 38°C the immobilised enzyme continues to rise but in much lower rate to 4. 00g/l per minute. However the mobilised enzyme decreases to 2. 38g/l per minute suggesting that it must have reached its peak at the temperature 35°C. At the temperature of 45°C both decrease to the values of immobilised enzyme to 2. 54g/l per minute and the mobilised to 1. 25g/l per minute. At the final temperature of 50°C both lactase enzymes continue to decrease. Immobilised lactase to 1. 0g/l per minute and the mobilised lactase to 0. 1g/l per a minute. Looking at the above and at the results you can see that the enzymes are working, as they should. As both immobilised and mobilised lactase enzymes are showing a peak, where they are working most efficiently (at there best) between 35°C and 38°C. This is how any enzymes work they work at a certain temperature at their best and work average or below average depending on the difference between their peak temperature and the certain temperature that they are working in.

But if the temperature is too high say above 60°C the enzyme will denature. But if too low the enzyme will work slower not as efficient as it is in its peek

temperature range where it works its best. After the temperature range of 35°C to 38°C the rate results of the enzymes start to decrease suggesting that the enzymes work the best at the temperatures mentioned earlier and the higher temperature after those temperatures will decrease the productivity of the enzyme eventually as the temperature increases the enzyme will denature.

References: •http://www. css. cornell. edu/compost/soda. html •http://www. 123helpme. com/view. asp? id= 148785 •http://www. exploratorium. edu/snacks/milk_makes-me_sick/index. html •http://www. chemistry. mcmaster. ca/~chem2o6/labmanual/expt11/2o6exp11. html •Biology book: Tim Kino, Micheal Reiss, Micheal Roberts 2001