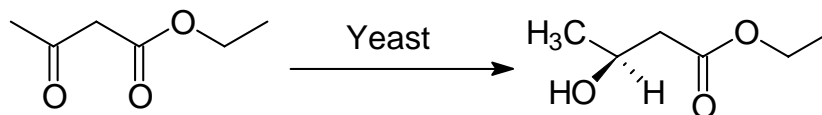


## Enzymatic Reduction: A Chiral Alcohol from a Ketone



The starting material for this experiment has two carbonyl groups, a ketone and an ester. Strong reducing agents such as lithium aluminum hydride ( $\text{LiAlH}_4$  or LAH) would cause reactions at both sites. The milder reducing agent, sodium borohydride ( $\text{NaBH}_4$ ), would react only with the ketone carbonyl. However, in this reaction of an achiral substrate with an achiral reagent to form a chiral product, it would be just as likely to form the ethyl **S**-3-hydroxybutanoate as the ethyl **R**-3-hydroxybutanoate. In other words, we would obtain the racemic mixture which, of course, would not exhibit any optical activity. This racemic mixture could be resolved to give the two enantiomers, one being dextrorotatory and the other levorotatory. In this experiment, we will use an enzyme found in ordinary baker's yeast to selectively prepare one of the enantiomers. Sucrose will be oxidized while the ethyl acetoacetate is reduced.

### Experimental

Using a 250 mL Erlenmeyer flask, dissolve 40 g of sucrose and 0.25 g of disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) in 150 mL of warm ( $35^\circ$ ) tap water. To this, add a package (8 g) of dry yeast and swirl to suspend the yeast throughout the solution. In about 15 minutes, when the fermentation is progressing nicely, add 2.5 g of ethyl acetoacetate. Put some cotton in the mouth of the flask, label the flask and store the flask in a warm place ( $30$ - $35^\circ$ ) until the next laboratory session.

Add about 10 g of Celite filtration aid, and remove the yeast cells by filtration with a 9 cm Büchner funnel. Wash the cells with 25 mL of water. Now saturate the filtrate with sodium chloride (to reduce the solubility of the desired product in water). Extract this saline solution five times with 25 mL portions of ether each time. (You need to thoroughly mix the two layers in the separatory funnel each time before allowing the phases to separate. Unfortunately, shaking too vigorously may lead to the formation of an emulsion at the interface. If this happens, a little methanol might break up the emulsion.) If a water layer is visible in the combined ether extracts, return it to the separatory funnel, and remove the water before proceeding to the next step.

The ether layer is then dried over anhydrous sodium sulfate. After around 15 minutes of drying, decant the ether solution into a tared distilling flask, and remove the ether by distillation. You should have around 1.5 to 2 g of product.

We will determine the optical activity of the product.