

The Aerobic Respiration Experiment

Introduction: All Eukaryotic cells require oxygen for aerobic respiration. Oxygen is readily used over the entire cellular respiration, where most of ATP is generated (oxidative phosphorylation). Glycolysis (occurs in the cytosol) starts off cellular respiration by oxidizing a glucose molecule into a series of two-carbon molecules (pyruvate), a net of four ATP, two NADH plus two hydrogen ions, and two water molecules. The pyruvate then enters the mitochondria and a hydroxyl group is removed creating carbon dioxide as a waste product. Acetyl CoA is then formed by the remaining two-carbon group and NAD⁺ is reduced to NADH. Acetyl CoA is formed when coenzyme A is attached, collecting bonds to create Acetyl CoA enters the Krebs cycle (citrate or citric acid cycle) and three-carbon atoms added to coenzyme A. FADH₂ and NADH (both electron-carrying) facilitate the electron-carrying cycle to mitochondria. The citric acid cycle has an output of four-carbon dioxide, two FADH₂, two H⁺, six NADH plus one hydrogen ion, and two coenzyme A. All the FADH₂ and NADH from the Krebs cycle and glycolysis transport electrons to the electron-transport chain. While the hydrogen bonds are pumped out the end of the chain, hydrogen ions get pumped into the inter-membrane space. These hydrogen ions are then pushed through ATP synthase, making a net of around 37-40 ATP (mostly generated at ATP produced during cellular respiration). This last step is called oxidative phosphorylation. The output of oxidative phosphorylation is around thirty-four ATP, two H₂O, one water molecule, and two H⁺. The spectrophotometer measures fluorescence as a measure of light. The level of fluorescence increases as increasing the concentration of a solution.

Purpose: The main purpose of this experiment was to study cellular respiration by using different chemical substances with a spectrophotometer to measure fluorescence. These substances change color during the electron-transport chain and during the citric acid cycle. The purpose was also to see the effect of having respiratory inhibiting substances on the rate and efficiency of cellular respiration under different conditions.

Procedure: Five conditions were set up like the following:

Tube	Enzyme	Substrate	Carrier	Inhibitor	Substrate
	None (Control)	GDH	β-Naphthol Buffer	None	None (none - Tubule for Steps 6 and 7)
1	200 ul	200 ul	4.0 ml	-	100 ul
2	200 ul	200 ul	4.0 ml	-	100 ul