

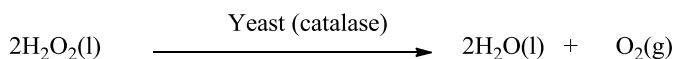
Biodecomposition of Hydrogen Peroxide (H₂O₂) in Water and in Organic Solvents Using *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (Fungi: Ascomycota)¹

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Abstract: The yeast-catalyzed decomposition of hydrogen peroxide was studied in water and in different organic solvents to discover the effect of their dielectric constant on the reaction. The reaction was most efficient in water producing a stoichiometric amount of molecular oxygen, O₂ (41.48 mL) from 3.71 g of hydrogen peroxide (3%) solution. Mixing water and ethyl alcohol (1:1, v/v) decreased the reaction's efficiency significantly, producing only 29.74 mL of O₂ from the same amount of hydrogen peroxide solution. The amount of O₂ decreased further to 20.19 mL when 100% ethyl alcohol was used. Carrying out the reaction in acetonitrile produced 24.90 mL of O₂. Ethyl acetate and tetrahydrofuran both produced about 19 mL of O₂, whereas 1-propanol and a 1:1 (v/v) mixture of water and dimethylsulfoxide produced about 15 mL of O₂. The reaction was least efficient in methanol and dimethylformamide, producing less than 3 mL of O₂ in both cases. No correlation between the dielectric constant of solvents and reaction's efficiency to produce O₂ was found.

Key Words: Yeast, *Saccharomyces cerevisiae*, hydrogen peroxide, catalase, organic solvents, oxygen

Microbial-mediated transformations have been widely used since ancient times in alcoholic beverages, bread-making, dairy products, and numerous other applications (Katz, 2012). The use of Baker's yeast, *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (Fungi: Ascomycota), as a reagent in organic synthesis has been well documented (Servi 1990, Csuk, and Glanzer 1991). In addition, organic chemists have also recognized the ability of Baker's yeast to transform unconventional substrates stereoselectively (Claudio 1990). When Baker's yeast is mixed with a solution of hydrogen peroxide, a vigorous evolution of oxygen occurs. This is because Bakers' yeast is exceptionally rich in the enzyme catalase that has high specificity and turnover rate in catalyzing the decomposition of hydrogen peroxide to produce oxygen and water.



Under appropriate aqueous conditions, the reaction is quantitative, making it a popular school experiment often used to investigate gas laws, gram-mole conversions and verification of the law of conservation of mass (Burness 1996, Bedenbaugh et al. 1988, Lugemwa 2013). The decomposition has an added

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advantage of employing a natural biological catalyst and relatively safe and readily available starting material that produce green and environment-friendly products after the reaction.

Yeasts catalyze a wide range of different types of reactions although they are commonly used for the asymmetric reduction of carbonyl groups and alkenes (Nakamura et al., 1991; Ohta et al. 1989). Most yeast-mediated reduction reactions have been conducted using fermenting yeast in an aqueous reaction system. Under these conditions, the yeast is actively growing (Figure 1), all of the biochemical pathways are operating, and, as part of the fermentation process, the substrate is converted into the human-desired product, usually with a high degree of stereospecificity. Although this often has worked well, the methodology has a number of inherent problems. The use of an aqueous environment for transformations involving organic substrates is undesirable, because of limited solubility in water. Product isolation is also a major challenge after the reaction has come to completion.

To use the molecular oxygen, O_2 , generated in the biodecomposition of hydrogen peroxide to study possible oxidations of organic substrates, different solvents as well as efficient promotion of the biodecomposition would be needed. Different solvents are important to ensure good solubility of the organic substrates. In this report, the yeast-catalyzed biodecomposition of hydrogen peroxide was carried out in commonly used organic solvents to study their effects on the production of O_2 from hydrogen peroxide catalyzed by Baker's yeast. Yeast catalase has been previously immobilized on beads and its action studied in acetonitrile (Dimcheva et al. 2004). Also, the reduction of keto-esters was carried out using yeast in different organic solvents (Rotthaus et al. 1997).

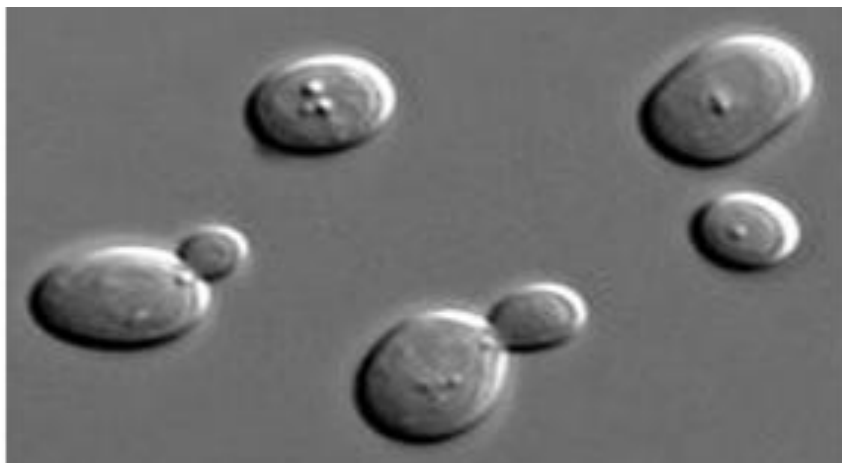


Figure 1. *Saccharomyces cerevisiae* yeast cells. Three smaller cells are in the process (two) or have just been budded off through mitosis. Image from Wikipedia, http://en.wikipedia.org/wiki/File:S_cerevisiae_under_DIC_microscopy.jpg

Methods

Red Star® Quick Rise™ Fast Acting dry yeast was obtained locally from a grocery store. Hydrogen peroxide (30%) and all solvents were of analytical grade from Sigma-Aldrich, St. Louis, Missouri, USA. Fresh hydrogen peroxide (3%) solutions made by diluting the 30% solution in different solvents were prepared each day the reaction was run. The concentration of hydrogen peroxide (3%) was checked by titrating with potassium permanganate. Yeast was kept in the refrigerator. The yeast- water mixture was prepared using yeast (1 g) in water (3 mL). For each solvent, the amount of the hydrogen (3%) solution in mL that produced stoichiometric amount of oxygen (40 mL) was used. Densities of different solution were used to determine the require volumes (Table 1).

Table 1. Decomposition of Hydrogen Peroxide, H_2O_2 , in water and in different organic solvents.

	Solution Density (g/ml)	Volume Used (mL)	Volume of O_2 (mL)	Reaction Time (min)
Water	0.94	3.93	41.48±1.5	25
Water-ethanol (1:1)	0.98	3.78	29.74±1.5	20
Acetonitrile	0.84	4.41	24.90±1.5	15
Ethyl Alcohol	0.83	4.47	20.19±1.5	25
Ethyl Acetate	0.99	3.74	19.41±1.5	10
Tetrahydrofuran	0.92	4.01	19.37±1.5	10
1-Propanol	0.89	4.17	16.32±1.0	10
Water/Dimethylsulfoxide (1:1)	1.06	3.51	15.20±1.0	20
1-4 Dioxane	1.03	3.61	14.55±1.0	20
Methanol	0.81	4.56	2.41±0.5	5
Dimethylformamide	1.03	3.59	0.09± 0.02	5

A gas collection apparatus, shown in Figure 2, was used to collect and measure the amount of gas the mixtures produced. A leveling bulb was attached to the burette and was used to equalize the pressure inside the collecting system. The set-up was first used by John H. Bedenbaugh et al. (1988) and later modified by James H. Burness (1996).

Hydrogen peroxide (3%) solution was placed into a 12 x 75 mm culture tube (4 in Figure 2). The yeast-water mixture (20 mL) was placed in a small plastic cup (5) and placed on top of the culture tube. The two were then carefully placed inside a 20 x 150 mm test tube with side arm (Figure 2).

The reaction was initiated by inverting the 12 x 75 mm culture tube and the small plastic cup, resulting in mixing the two solutions inside the 20 x 150 mm test tube with side arm. The test tube was gently shaken once every minute until the visible evolution of gas stopped. Each run was carried out in triplicate.

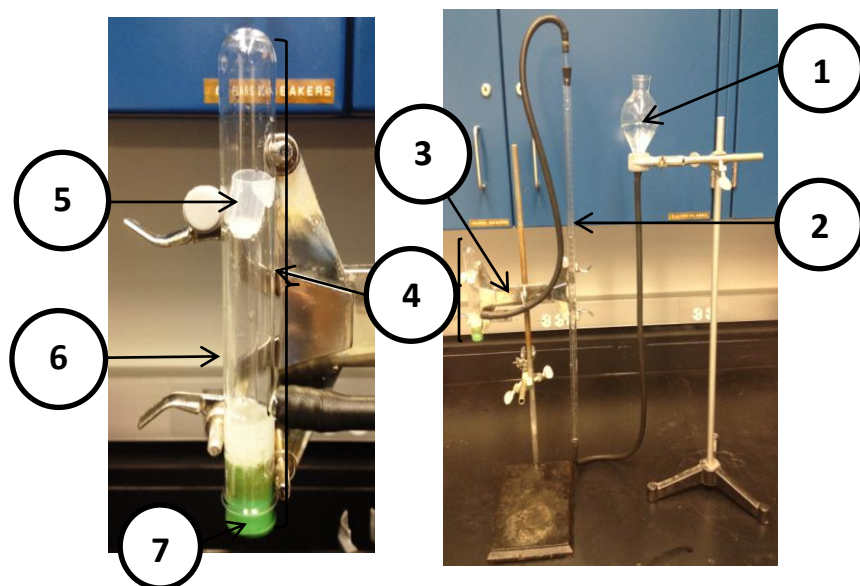


Figure 2. Gas collection apparatus. 1. Leveling bulb. 2. Burette. 3. Test tube clamp. 4. 12 x 75 mm culture tube, for the H_2O_2 /solvent mixture. 5. Small plastic cup, for the yeast-water mixture. 6. 20 x 150 mm test tube with side arm. 7. Rubber stopper.

Results and Discussion

The yeast-catalyzed decomposition of hydrogen peroxide was found to proceed most efficiently in water (Table 1). The decomposition was completed in 25 minutes, producing a stoichiometric amount of oxygen (41.48 mL). Water is the natural environment for the organism and its enzyme, and, not surprisingly, the reaction proceeds most effectively in an aqueous medium. The decomposition proceeded well in acetonitrile, an aprotic polar solvent, producing 24.90 mL of oxygen after 15 minutes. Longer times did not result in additional decomposition to yield more oxygen. Ethyl acetate and tetrahydrofuran proceeded with the same efficiency producing about 20 mL of oxygen and the reaction stopping after 10 minutes. Ethyl alcohol too produced 20.19 mL of oxygen, but the reaction took longer (25 minutes), indicating that although the reaction seems to proceed faster in tetrahydrofuran and in ethyl acetate than in ethyl alcohol, those two solvents that promote a faster reaction could also be denaturing the enzyme or killing the organism faster. One possible explanation is that the active site of the enzyme catalyzes the decomposition well in those solvents, but at the same time, other parts of the enzyme may be affected because of denaturation. The decomposition was more efficient in a 1:1 (v/v) water/ethyl alcohol mixture than in pure ethyl alcohol.

The reaction took 20 minutes to come to completion and produced 25 mL of oxygen. The reaction in 1-propanol, a 1:1 (v/v) mixture of water/dimethylsulfoxide, and 1,4-dioxane generated about 15 mL of oxygen in each case. However, the reaction in 1-propanol came to completion two times faster than in the other two solvents. The reason for this is not clear, but could also be caused by the denaturation of the enzyme inside the yeast. It is possible that the enzyme works faster in 1-propanol, but at the same time the same solvent is destroying part of the enzyme, or is toxic to the whole organism. The worst solvents in promoting the decomposition were methanol and dimethylformamide, yielding 2.4 mL and 0.09 mL respectively. Overall, no correlation was found between reactivity and the dielectric constants (E_T) of solvents. The reaction was most efficient in the most polar solvent, water ($E_T = 80$), but also very inefficient in methanol another polar protic solvent ($E_T = 33$). The bio-decomposition in ethanol ($E_T = 25$) was ten times more efficient than in methanol. The reaction was efficient in acetonitrile a polar aprotic solvent ($E_T = 38$), but did not work well in dimethylformamide also a polar aprotic solvent ($E_T = 37$). Dimethylsulfoxide, another polar aprotic solvent ($E_T = 47$) did not work as a pure solvent. However, a dimethylsulfoxide water mixture (1:1, v/v) promoted the decomposition well. An attempt to carry out the reaction in the more volatile ethers (diethyl ether, tert-butylmethyl ether, and di-isopropyl ether) was not successful. It was difficult to get an initial reading as the solvent evaporated instantly and the system could not come to equilibrium. Solvents like hexane and cyclohexane were not tasted, because their ability to dissolve organic substrates would be limited when coupling the nascent oxygen produced and its ability to oxidize organic substrates *in situ*. The use of the more toxic solvents, such as benzene and toluene was also not desirable.

Using yeast as the biological agent to study the decomposition of hydrogen peroxide was challenging because the observed effects could have resulted from the solvents affecting the whole unicellular organism or, specifically, the enzyme, catalase. Using a purified enzyme could allow us to discover a better explanation of the observed effects. The decomposition in some organic solvents proceeded well, and the activity of the enzyme increased when water was combined with those solvents.

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