

KINETICS OF DISSOLVED OXYGEN CONSUMPTION AND DEOXYGENATION OF
PINEAPPLE JUICE AND MODEL SOLUTIONS USING A THIN FILM ENZYME
REACTOR

By

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LIST OF ABBREVIATIONS

DO	Dissolved oxygen
GOx	Glucose oxidase
GOx-Cat	Glucose oxidase- Catalase
L-AA	L-Ascorbic acid
PPD	poly- <i>o</i> -phenylenediamine
PBD	Packed bed reactor

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KINETICS OF DISSOLVED OXYGEN AND DEOXYGENATION OF PINEAPPLE JUICE
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Dissolved oxygen (DO) affects the quality of fruit juices by reacting with ascorbic acid and flavor components. In this study experiments were run at 5.0, 13.0, 21.5, 30.5 or 40.0 °C for both DO measurement and Ascorbic acid (AA) measurement. First order, second order and 0.5 order kinetic mechanisms were considered. First order model fit better initial consumption of DO in AA solutions and pineapple juices. First order reaction rate constants ranged between 1.03×10^{-5} to $2.97 \times 10^{-4} \text{ s}^{-1}$ for 5°C and 40 °C, respectively for 28 mM AA solutions and 9.58×10^{-6} to $1.08 \times 10^{-4} \text{ s}^{-1}$ for 13°C and 40 °C, respectively for 2.8 mM AA solutions and between 8.89×10^{-6} to $4.75 \times 10^{-5} \text{ s}^{-1}$ for 13°C and 40 °C, respectively for pineapple juices. In both AA solutions and pineapple juices the reaction rate of DO consumption followed Arrhenius behavior. The activation energy (E_a) for DO consumption was calculated as 67.7 ± 4.2 , 67.12 ± 6.72 and $48.62 \pm 2.6 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ for 28 mM, 2.8 mM AA solutions and pineapple juices respectively. Temperature did not show a significant difference in decrease in the rate of AA degradation between 13°C and 40°C.

An enzyme reactor consisting of Glucose oxidase - Catalase complex immobilized in electrochemically generated poly-*o*-phenylenediamine thin films deposited on the

interior wall of a platinized platinum tube was proposed for deoxygenation of fruit juices. Reactors with GOx concentrations of 10 and 25 g L⁻¹ showed better operational stability compared to 50 g L⁻¹ where film detachment was observed. Increasing the retention time from 127.4 s to 382.4 s increased the % of DO removal from 63.6% to 91.6% in a 12-cm reactor. The effect of pH on reactor performance is negligible. We did not observe any effect of catalase (equivalent to two times GOx activity) on the performance of reactor. However, when increasing the catalase concentration from two times to five times the activity of GOx we did not observe any decrease in oxygen concentration. In both model solutions and pineapple juices approximately 90% of the DO was removed using a 12-cm enzyme reactor at a flow rate of 0.025 mL min⁻¹ with a retention time of 6.4 min.

CHAPTER 1 INTRODUCTION

Dissolved oxygen in the fruit juices causes number of problems. Dispersed oxygen is easier to remove than dissolved oxygen. The most stable and abundant form of oxygen is the molecular or diatomic triplet oxygen.

Solubility of Oxygen

The solubility of oxygen in a solution is mainly dependent on three factors (temperature, pressure and solutes concentration). The solubility decreases with increasing temperature, decreasing partial pressure and with increase in solute concentration. The solubility of a gas dissolved in a liquid is described by Henry's law it states that the concentration of a gas dissolved in a liquid is proportional to the partial pressure of the gas in the vapor phase:

$$X_A = \frac{P_A}{H(T)} \quad (1-1)$$

where X_A , P_A and $H(T)$ are mole fraction of gas A in the liquid phase, partial pressure of gas A in the vapor phase and Henry's constant respectively (Gill and Menneer 1997).

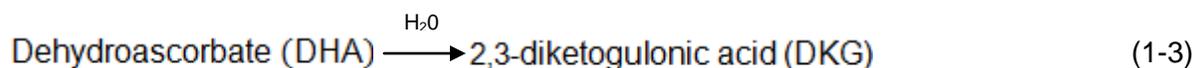
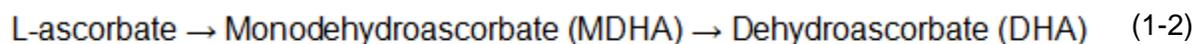
Effect of Dissolved Oxygen on Fruit Juices

Dissolved oxygen plays a vital role in the deterioration reactions of citrus products such as degradation of ascorbic acid and thus causes the non-enzymatic browning of juices during the storage which results in the formation of off-flavors (Rassis and Saguy 1995). Degradation of ascorbic acid is one of the major causes of color and quality changes during storage. Falade and others (2004) reported 16.25% and 16.67% ascorbic acid loss in sweetened Julie and Ogbomoso mango juices at 25°C after 12

weeks. The loss of vitamin C in sweetened mango juices was attributed to both aerobic and anaerobic reactions. Rate of ascorbic acid degradation during storage increased with increasing temperature, presence of copper, iron and alkali, increased light exposure (Mack and others 1976; Singh and others 1976) and differences in pH. Difference in pH effect the number of ascorbic acid oxidation steps (1 or 2 steps). A synergistic relationship, resulting in increased ascorbic acid degradation was reported between light and temperature. However, some contradicting data has been reported about the rate of ascorbic acid degradation in the literature. Singh and others (1976) reported ascorbic acid degradation in liquid food as second order reaction. Mack and others (1976) reported oxygen uptake as a first order rate during the first 24 h in the same solution. Various studies on model solutions and citrus juices reported ascorbic acid degradation as first order, zero order and second order kinetics; these contradictions in the results are due to the variations in the concentrations of DO. Garcia-Torres and others (2009) reviewed the interaction of dissolved oxygen consumption with various food components and reported that direct aerobic oxidation of L-AA indirectly affects color and aroma profile.

L- ascorbic acid (L-AA), degradation occurs by two consecutive or parallel pathways aerobic and anaerobic (Kennedy and others 1992; Johnson and others 1995; Manso and others 2001b; Baiano and others 2004). In the aerobic pathway, ascorbic acid in aqueous solution is easily oxidizes to mono dehydroascorbate (MDHA) also called ascorbate free radical. MDHA can be reduced back to L-AA or two MDHA molecules can produce L-AA and DHA. Dehydroascorbic acid (DHA) is unstable and

undergoes irreversible hydrolytic ring cleavage to produce 2, 3 diketogulonic acid in aqueous solution (Equation 1-2 and 1-3).



Under anaerobic conditions, L-Ascorbic acid is degraded to furfural and rate is slower than aerobic degradation (Baiano and others 2004).

Methods of Deaeration

To prevent the deleterious effects due to DO, fruit juices are often deaerated prior to pasteurization. Deoxygenation is accomplished by vacuum flash deaeration, gas sparging, oxygen scavengers, membrane deaerators and adding antioxidants using enzymes. Vacuum deaeration is the most commonly used method in the citrus industry. However, in this method essential oil and flavor compounds are also removed along with oxygen (Braddock 1999). Gas sparging consists of displacing the DO with another gas such as nitrogen or carbon dioxide. Early studies on methods of deaeration indicate that in orange juice 99% of DO was removed using nitrogen sparging compared to 77% DO removed by vacuum deaeration (Joslyn 1961) but significant amount of volatiles are removed in this process too. Jordan and others (2003a) reported that the greatest losses in concentration of volatiles occurred during industrial deaeration. Membrane deaerators are most commonly used for water and waste water treatment and some other food products, but their application to fruit juices is limited (Cole and Genetell.Ej 1970) which may be due to the interference of pulp with the membrane. Garcia-Torres and others (2009) reviewed different deaeration methods and reported that vacuum

deaeration and gas sparging suitable to remove oxygen in fruit juices but they also removed important volatile aroma compounds.

Vacuum Deaeration

Vacuum deaeration is based on the reduction of pressure of the gas above the juice. Vacuum flash deaeration is the most commonly used method in the citrus industry. In this process, the juice is preheated to 50-60°C and sprayed inside a vacuum vessel, where the juice will flash (boil). Oxygen and volatile compounds are separated from the juice. The pressure in the vacuum chamber and the inlet temperature are adjusted so that the inlet temperature is 2-5°C above the boiling point of the orange juice at that pressure (Ringblom 2004).

Gas Sparging

Gas sparging or bubbling consists of displacing the dissolved oxygen with another gas such as nitrogen, helium or carbon dioxide. The partial pressure of oxygen in the vapor phase is reduced by displacing the oxygen with another gas such as N₂ or CO₂. The gas can be bubbled into the liquid, meet in countercurrent with the liquid, or the liquid can be sprayed into a vessel filled with gas. The height of the chamber, size of bubbles of gas and flow rates of gas and liquid determine the rate and extent of oxygen removal. The disadvantage of this deaeration process is that, like vacuum deaeration, it also removes flavor volatile compounds (Jordan and others 2003b). For this reason new techniques such as membrane and enzymatic deaeration are being developed.

Membrane Deaerators

Membrane deaerators are widely used to remove oxygen from water, beer, wine and other particle-free product. Membrane deaerators consist of several hollow membrane fibers knitted into a fabric and wrapped around a center tube. Liquid flows

into the center tube and is forced to pass radially through the membrane, vacuum or a swept gas are applied in order to remove the oxygen from the liquid.

Cole and Genetell.Ej (1970) reported that 96% of DO was removed from water using hollow fiber membranes. Jiahui and others (2008) developed a hollow fiber membrane system that removes DO in boiler feed water. The membrane was made using hydrophobic polypropylene. The outer diameter of fiber was 300 µm and the thickness was 100 µm. The efficiency of deoxygenation decreased after a long period of time. This was attributed to the membrane fouling which occurred due to organic matter and aluminum silicate in the feed water. There is a large body of research available on membrane deaerators and their application to water and pieces of equipment are commercially available. However, the use of these deaerators in the fruit juices was not successful. This may be due to the interference of pulp with the membrane.

Enzymatic Deaeration

Glucose oxidase (GOx, EC: 1.13.4), which was discovered in 1928 by Muller in *Aspergillus niger* and *Penicillium glaucum*, catalyzes the oxidation of β-D-glucose to D-glucono 1,5-lactone and hydrogen peroxide in aerobic conditions as shown in Equation 1-4. Catalase (Cat, EC: 1.11.1.6), decomposes hydrogen peroxide to water and oxygen as shown in Equation 1-5.



Enzymatic deaeration of juices using GOx-Cat in solution has been reported by (Sagi and Mannheim 1990; Parpinello and others 2002). For each mole of oxidized glucose the enzymatic method removes half a mole of oxygen (Sagi and Mannheim

1990). The advantage of this system is that glucose is present in large excess with respect to DO in juices so there is no need to add any substrate. Some other commercial applications using GOx-Cat in food products include desugaring of eggs, production of reduced alcohol wine and prevention of browning in apple and pear purees.

GOx-Cat has been used to remove glucose from egg white during the commercial preparation of egg white powder in order to prevent non enzymatic browning during processing and storage (Sankaran and others 1989). GOx 92.3 g t⁻¹ at 25-30 °C, pH 5.5-7 was able to remove 95% of glucose from egg white and effectively suppressed the Maillard reaction during processing and storage. They also found that the desugared egg white maintained its initial functional characteristics with better smell and mobility than untreated egg white.

Pickering and others (1998) observed low pH of grape juice was a limiting factor in the production of reduced alcohol wine using GOx-Cat. Optimizing the process resulted in 87% conversion of glucose to D-gluconic acid and was achieved by raising the pH of juice by adding calcium carbonate. Parpinello and others (2002) studied use of GOx-Cat system to prevent non enzymatic browning in apple and pear purees and found that GOx was able to remove 99% oxygen in apple and pear purees which helped in preventing oxidation and browning. They also observed that the ascorbic acid prevented browning to a larger extent than any other chemical. Ascorbic acid enhanced the activity of GOx-Cat system in preventing browning in apple juice, suggesting a synergistic effect. Grape juice treated with GOx-Cat and subjected HHP at 600 MPa improved the sensory properties and also helped in color stabilization of juice (Castellari

and others 2000). Ough (1975) reported that GOX-Cat effectively removed DO from white table and rose wines. Hydrogen peroxide was reduced by sulfur dioxide rather than catalase.

Immobilization of Enzymes

Advantages of Immobilized Enzymes

Enzymes are catalysts that increase the rate of reaction without being unchanged. They do this by lowering the activation energy of reactions. Although the cost of major processing enzymes has decreased considerably, enzyme costs are still important in the food and pharmaceutical industries. Enzymes can be chemically or physically immobilized to an insoluble support so that it can be physically reclaimed from the reaction medium thus decreasing enzyme costs. Like all proteins enzymes are affected by temperature, pH and inhibitors. Immobilization often mitigates such effects increasing the reusability of enzymes and further decreasing operation costs. The first type immobilization technique based on adsorption was developed by Nelson and Griffin in 1916. After that several different immobilization techniques were developed. Up to now more than 5,000 publications and patents are available on different enzyme immobilization techniques.

Different supports and techniques can be used for immobilization of enzymes. One of the important property is the support material should have high affinity for proteins. Availability of reactive functional groups for direct reaction with proteins or for chemical modifications and non-toxicity (Agullo and others 2003). The selection of suitable enzymatic preparation depends on enzyme properties and the purpose of its application. Yeast β -galactosidases are generally used for the hydrolysis of lactose in milk and sweet whey whereas fungal β -galactosidases are used for acidic whey

hydrolysis because fungal enzymes are more thermostable than yeast enzymes. The supports and techniques are chosen in such way that maximum enzyme activity, stability and durability are achieved.

Limitations of Immobilization

Immobilization of enzyme has several advantages. However, some limitations are also associated with immobilization. After immobilization enzymes are restricted in movement so decrease of enzyme activity has been reported compared free enzymes. However, the extent of decrease depends mainly on the immobilization method and the source of enzyme. One of the easiest ways of immobilizing enzymes is through adsorption however, the main limitation is leakage or desorption of the enzyme from the matrix.

Applications of Immobilized Enzymes

There are numerous applications available using the immobilization of enzymes. Immobilized enzymes are widely used in the different fields. One of the most important applications of immobilized enzymes is in the production of high fructose corn syrup. Production of cheese using immobilized enzymes has been considered as promising step for the rationale use of rennin. Grosova and others (2008) reported application of immobilized β -galactosidase in the hydrolysis of milk and whey lactose. Baticz and Tomoskozi (2002) developed an immobilized cholesterol oxidase reactor for determination of total cholesterol content in foods. Parpinello and others (2002) Pickering and others(1998) reported different applications immobilized glucose oxidase-catalase system for desugaring of eggs to prevent non enzymatic browning, production of reduced alcohol wine and prevention of browning in apple and pear purees. Immobilized enzyme biosensors are tested for determination of Ascorbic acid in human

serum using ascorbic oxidase immobilized in poly o - phenylenediamine film. There are some applications using immobilized enzymes in active packaging. Kothapalli and others (2007) studied GOx in low-density polyethylene using UV polymerization.

Methods of Immobilization

Several methods of enzyme immobilization have been developed aiming at preventing loss of enzyme activity and maximizing activity. A good understanding of the active site of the enzyme is often critical in the selection of the method of immobilization. Several immobilization techniques are available based on the application. These include adsorption, covalent bonding by tethering or cross-linking, encapsulation, and entrapment in gels or polymers.

Adsorption

Adsorption is the simplest and oldest way of immobilizing the enzyme. The enzyme is mixed with the support material under appropriate conditions and is adsorbed onto the support material usually through hydrogen bonding, hydrophobic interactions; Several support materials are available for immobilization like cellulose, collagen, calcium carbonate etc. However, this type of immobilization is dependent on experimental conditions like pH, temperature, ionic strength. Therefore, changes in these conditions can result in enzyme desorption of the support material. For example, if ion-ion interaction is predominant with very little hydrogen bonding, ionic strength changes then a small shift in pH could result in the desorption of the enzyme.

Covalent Bonding

This is one of the most intensely studied immobilization technique. Here formation of covalent bond between enzyme and the support matrix takes place. Typically enzymes covalently bind to a reactive group on the support material (typically amino,

carboxy or hydroxy-derivatized surfaces) through the reactive groups on side chains of its amino acids or with terminal amino and carboxyl groups of the polypeptide chains. Also, inter molecular cross-linking of enzymes by bi functional or multi functional reagents have been widely used. The most common reagent used for cross linking is gluteraldehyde. Cholesterol oxidase was immobilized on amino modified Kieselgel 100 polymer beads by cross linking with gluteraldehyde for the determination of total cholesterol in food by flow injection analysis.

Gel Entrapment

Entrapment of enzymes into the semi permeable gel or enclosing the enzyme semi permeable polymer membrane. Polyacrylamide is one of the most commonly used matrix for entrapment of enzymes. A number of procedures for the entrapment of enzymes in polyacrylamide gels have been published.

Polymer Entrapment

Conventional procedures for immobilizing enzymes like cross-linking, covalent bonding and entrapment in gels or membranes suffer from a poor reproducibility and a poor spatially controlled deposition. The immobilization of enzymes in electrochemical polymers is gaining importance. Unlike, conventional immobilization methods, the electrochemical formation of polymer layers of controlled thickness gives a reproducible and non manual procedure for sensor fabrication. The electrochemical method involves the entrapment of biomolecules in organic layers during their electro deposition on an electrode surface. Most of the polymers used in electrochemical generation are conducting polymers such as polyacetylene, polythiophene, polyaniline, polyindole and polypyrrole. In comparison to the physical entrapment of enzymes within polymer films such as polypyrrole, polythiophene, polyacetylene or polyaniline, this approach

preserves a better access of substrate to the immobilized molecules and facilitates macromolecular interactions.

Encapsulation

Microencapsulation focuses upon maintaining the solution environment around the enzyme rather than physical or chemical forces necessary for immobilization. Formation of polymeric membrane around an enzyme solution to make a microsphere requires a great deal of technology. The diameter of the microspheres can range from several microns to several thousand microns and the membrane thickness can go from hundreds of Angstroms to several microns.

Immobilized Enzyme Reactors

The objective of an enzymatic reactor is to allow enzyme and substrate to come into contact for a sufficient period of time for reaction to take place; after that enzyme can be recovered at the end of the reaction and further the processed product is not contaminated with enzyme (Roig and others 1987). Immobilization gives more surface area available for reaction per unit volume thus improving the rate of liquid to solid mass transport of the substrate. The selection of proper reactor system depends on the type of immobilization and the type of process (Roy and Gupta 2003). Some enzyme reactors developed for food applications are discussed below

Packed Bed Reactor (PBR)

In a packed bed reactor the immobilized particles are held in a column and substrate is pumped through in plug flow mode. The flow rate profile across a transverse cross section of the column is perfectly flat. Systems like this are called plug flow reactors. The flow of substrate can be either from top or bottom and the column can be either vertical or horizontal. The main disadvantage of packed bed reactor is that

temperature or pH is not controlled properly especially in reactors with diameters greater than 15 cm. Zhou and Chen (2001) and others immobilized β -galactosidase on graphite surfaces by gluteraldehyde. They used this PBR for continuous hydrolysis of lactose in skim milk solution. At flow rate of 7 mL min^{-1} 90% conversion of lactose was achieved within a residence time of 15 minutes. Ovsejevi and others (1998) studied the immobilization of β -galactosidase on to activated agarose gel by thiosulfinate. The mini reactor was used for the lactose hydrolysis. Whey permeate at a flow rate of 7 mL h^{-1} was fed continuously and a 90% conversion was observed.

Illanes and others (1999) designed an immobilized enzyme reactor using immobilized lactase and glucose isomerase in sequential packed bed reactor for continuous production of fructose syrup from whey permeates. Baticz and Tomoskozi (2002) developed an immobilized enzyme reactor to determine the total cholesterol content in foods using cholesterol oxidase. The Enzyme was immobilized on Amino modified Kieselgel 100 polymer beads by cross linking with gluteraldehyde. They reported that the immobilized reactor was stable for two months. The diameter, length of the reactor and the flow rate were optimized to get the best possible results.

Fluidized Bed Reactor (FBR)

In a fluidized bed reactor the immobilized enzyme particles are fluidized. The particles become suspended in the substrate stream. Immobilized enzyme particles density should be sufficiently high to prevent them from being blown out of the reactor. Otherwise larger diameter particles have to be used (Roy and others 2000). This reactor is quite efficient at mixing catalyst with the substrate. Coughlin and others (1978) designed the first fluidized bed reactor for whey hydrolysis in pilot plant. The enzyme β -galactosidase was adsorbed on porous alumina and cross linked with gluteraldehyde.

Roy and Gupta (2003) designed a FBR by immobilizing the enzyme on epichlorohydrin-activated cellulose beads. The reactor with recirculation of substrate was used for hydrolysis of lactose in milk whey and whole milk. Ninety four percent of the conversion of the lactose in milk whey was observed. However, the conversion in whole milk did not cross 60% this was attributed to the fat molecules that impairs the performance of the fluidized bed. The main disadvantage of this type of reactors is that they are difficult to scale up so their use is restricted to small scale but for high cost products (Roy and others 2000).

Table 1-1. Immobilized enzyme reactors.

Type	Application	Advantages	Limitations	References
Packed bed reactor	To determine the total cholesterol in foods. Lactose hydrolysis in milk. Continuous production of fructose syrup from whey permeate.	The immobilized enzyme reactor values are in comparable with GC values. These reactors are being used in the industry.	Temperature or pH is not controlled properly especially in reactors of >15cm diameter.	(Zhou and Chen 2001) (Baticz and Tomoskozi 2002) (Ovsejevi and others 1998) (Baticz and Tomoskozi, 2002) (Illanes and others 1999)
Fluidized bed reactor	Hydrolysis of lactose in milk whey	Ninety four percent conversion of lactose was observed in milk whey.	Density of immobilized enzyme particles should be high or diameter should be large. Difficult to scale up. Hydrolysis of lactose did not cross 60% in whole milk.	(Roy and others 2000) (Coughlin and others 1978) (Roy and Gupta 2003)

Measurement of Dissolved Oxygen

Introduction

The US geological survey (USGS) standard field methods to measure dissolved oxygen are amperometric, luminescence, winkler titration, and colorimetric methods. Several limitations were associated with some of these methods. Industry standard technique for measurement of DO is conventional Clark-type electrode (amperometric method). Limitations associated with electrochemical probe include drifts in calibration due to oxygen consumption by the electrode, need for turbulent flow to prevent the formation of concentration gradients and electrical interference. The most recently developed methods are solid-state luminescence methods based on fluorescence peak quenching or multi frequency phase shift. The most common fluorophores are ruthenium or platinum complexes (Lee and Okura 1997) because they are excited and emit at wavelengths in the visible light range and they have high sensitivity to oxygen and large stokes shifts. Ruthenium complex based sensors are the most commonly used and are used for aqueous liquids and vapors. There are some commercially available ruthenium based sensors that can be used for the determination of DO in organic liquids and vapors.

Another way of measuring oxygen using luminescent based sensor system is using phase modulation techniques. The phase shift between the exciting and the emitted light source is correlated to DO concentration (Barnes and others 1990; Jiang and others 2002) Phase measurements are more stable and essentially independent on the luminescence dye concentration and other factors which affect the intensity signal (Ogurtsov and Papkovsky 1998).

Fiber Optic Oxygen Sensors

The principle of operation of this type of sensors is the fluorescence quenching of a fluorophore. When a fluorophore in its excited state collides with oxygen molecules then the energy is transferred to the oxygen molecule in a non radiative transfer, decreasing fluorescence intensity. The higher the number of collisions, the greater is the fluorescence quenching.

The fluorophore is usually a ruthenium or platinum complex. Ruthenium complex based sensors are the most commonly used and are used for aqueous liquids and vapors. Optic sensors having Platinum complex as a fluorophore is used for the measurement of low levels of DO. These sensors have high sensitivity compared to other optical sensors. There are some commercially available ruthenium based sensors that can be used for the determination of DO in organic liquids and vapors.

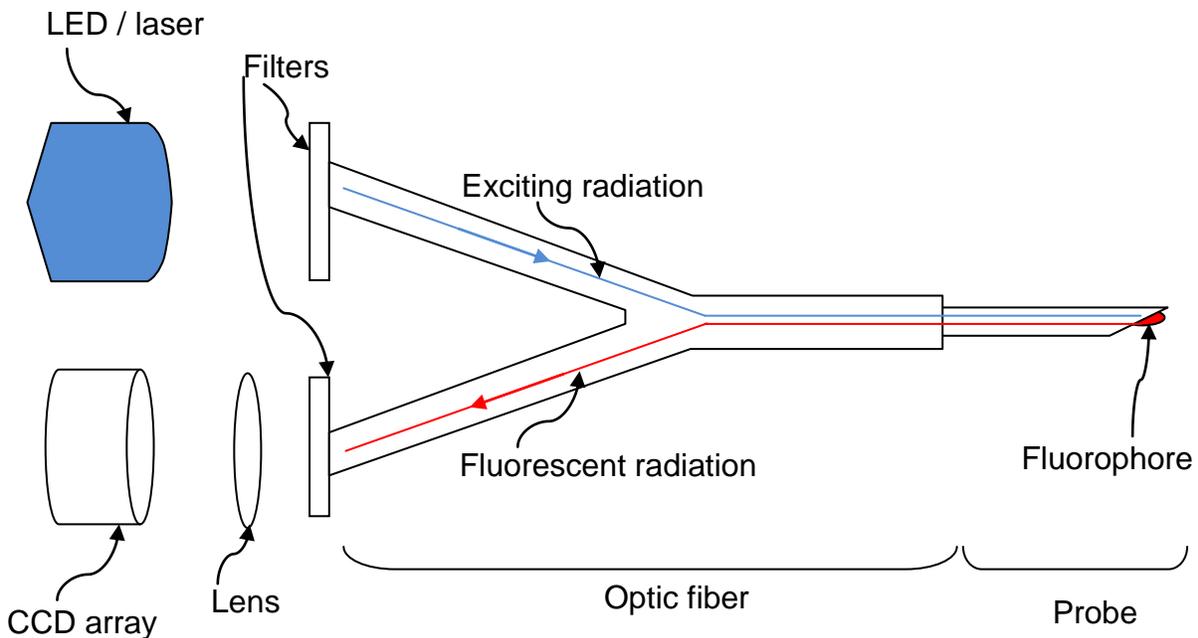


Figure 1-1. Schematic diagram of an optical oxygen sensor

Working

The pulsed blue LED (laser emission diode) sends light at 475 nm through one optical fiber. The light excites fluorophore which is immobilized at the tip of the probe. The excited Ru complex fluoresces and emits energy at 600 nm; The Pt complex energy is emitted at 650 nm. The emitted light is collected through a second optical fiber to the spectrometer. An Analog to digital converter converts this analog data to digital data and is typically collected and displayed by a computer program.

Calibration

Calibration of optical sensors is based on the following Stern–Volmer equation

$$\frac{I_0}{I} = 1 + K_S [O_2]$$
$$K_S = k\tau_0 \tag{1-6}$$

Where I_0 and I are the luminescence intensities in the absence and presence of oxygen respectively, K_S is the Stern – Volmer constant, $[O_2]$ is the oxygen concentration, k is the diffusion – dependent bimolecular quenching constant and τ_0 is the excited-state lifetime of the fluorophore in absence of O_2 . The plot of I_0/I vs. $[O_2]$ is a straight-line with slope equal to K_S and an intercept of 1 (McDonagh and others 1998). However sometimes, Stern Volmer plots are non linear due to microscopic heterogeneity in the structure of the sample matrix. This heterogeneity is explained by the presence of sites with different quenching constants, called, quencher-easy and quencher-difficult accessible sites (Shin and others 2002). When Stern – Volmer plot is not linear, a second order polynomial calibration is recommended

$$\frac{I_0}{I} = 1 + A * [O_2]^2 + B * [O_2] \tag{1-7}$$

Where A and B are temperature dependent coefficients. B is analogous to the K_s constant in Stern – Volmer equation. When the probe does not have an overcoat it fluoresces in all directions. In the presence of a reflecting surface or backscattering medium the intensity of the emitted light is greater than in transparent, non reflective media. So when calibrating a probe (not having overcoat) standards should be in the same state of matter as sample and also must have same refractive index as sample. So one cannot calibrate in gas and correlate it to liquids.

Current Research

Optical oxygen sensors consist of an oxygen sensitive dye entrapped in a matrix with a high permeability to oxygen. The fluorophore is deposited using sol-gel immobilization technique. The use of sol-gel immobilization has several attractive features these include ambient processing conditions, chemical inertness and stability of the sol-gel, high optical transparency, ease of use and the ability to tailor the structure of the matrix through control of the processing parameters (Campbell and Uttamchandani 2004). Various precursor compounds are available for preparation of sol-gel structures. The most common ones are tetraethoxysilane (TEOS) and tetramethoxysilane (TMOS). However ORMOSILs (organically modified silicates) are a class of precursors that have received increasing attention over the years. These compounds are of the form $RSi(OR)_n$, where R is an alkyl group covalently attached to the silicon and is not present in the non modified precursors. Alkyl groups in ORMOSILs have poor affinity towards water. This hydrophobicity has been the reason for increasing response of ORMOSILs than for non-mediated precursors such as TEOS since oxygen permeation is generally greater in hydrophobic media. The ORMOSILs also stabilize very quickly which makes them to use in harsh environments soon after preparation.

Stability and response time of the sensor depends on both the quenching rate and matrix characteristics. For example, density, viscosity and hydrophobicity.

Many Luminescent dyes have been tested as oxygen sensing probes. However Ruthenium and platinum complexes (Lee and Okura 1997) are the most commonly used because of visible light absorption, high sensitivity to oxygen and large Stoke's shifts. In an attempt to increase the sensitivity, photostability and response time of the sensors several methods have been tried over the years and are broadly classified into three categories.

1. Modification of the sensor system: Klimant and wolfbeis (1995) proposed optical isolation of the sensing layer to minimize photo bleaching of the dye. Black silicone, black teflon and titania were used as isolation layers which made the sensor more photo stable than those with only a sensing layer.
2. Modification of the existing oxygen sensitive dyes: Earlier in optical oxygen sensors the matrix was doped with ruthenium complexes since this particular dye has a long excited lifetime (5.3 μ s), a high luminescence quantum yield and a high oxygen quenching efficiency. However there is an increasing attention towards pt based sensors. Pt complexes are easily excited using compact and low-cost LED light sources. Furthermore the fluorescence wavelengths of Pt complexes are well separated from the excitation wavelength and hence the influence of excitation light source can easily be minimized. Ru based sensors emit energy at 600 nm. Pt complex based sensors emit energy at 650nm. Watkins and others (1998) reported that Ru(dpp)_3^{2+} immobilized in TEOS sol-gel has sensitivity of 12 and response times 3.5 s when switching from 0% O_2 to 100% O_2 and 30 s from 100% O_2 to 0% O_2 Tang and others (2003) reported that Tris (4,7 ϕ -diphenyl-1,10 ϕ -phenanthroline)- Ruthenium (II) chloride pentahydrate (Ru(dpp)_3^{2+}) immobilized in Octyl-triEOS/TEOS sol-gel has a nitrogen-to-oxygen intensity ratios ($I_{\text{N}_2}/I_{\text{O}_2}$) of 16.48 where as (Yeh and others 2006) reported that oxygen sensitive dyes Platinum tetrakis pentafluorophenylporphine (PtTFPP) and platinum octaethylporphine (PtOEP) immobilized in same matrix Octyl-triEOS/TEOS sol-gel has $I_{\text{N}_2}/I_{\text{O}_2}$ of 22 and 47 respectively. This shows that modification of existing oxygen sensitive dyes with Pt complexes has resulted in increased sensitivity and photostability.
3. Modification of the support matrices: In general, the support matrix of an optical sensor not only immobilizes the dye, but also helps in oxygen to penetrate into thin film to react with the sensitive dye. Different matrixes yield different oxygen diffusion rates, and hence have a direct influence on the quenching efficiency of the indicator by the oxygen. Ru(dpp)_3^{2+} immobilized in support matrices TEOS

sol-gel, Octyl-triEOS/TEOS sol-gel and n-propyl-TriMOS/TFP-TriMOS have sensitivities of 12, 16.48 and 35 respectively.

Yeh and others (2006) reported that Fluorophores PtTFPP and PtOEP immobilized in Octyl-triEOS/TEOS sol-gel have sensitivities of 22 and 47 respectively. Chu and Lo (2007) reported that PtTFPP and PtOEP immobilized in n-propyl-TriMOS/TFP-TriMOS have sensitivities of 68.7 and 82.5 respectively. From the above studies it is understood that the change in the support matrices has improved the sensitivity and response time of the sensors.

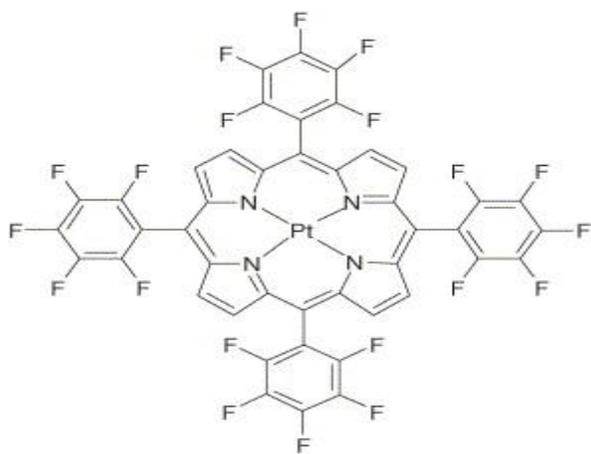


Figure 1-2. Chemical structure of PtTFPP

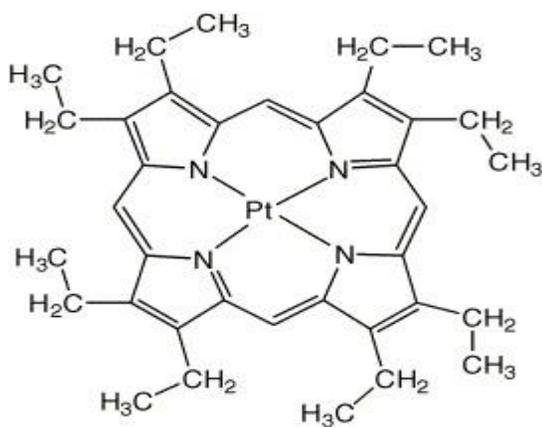


Figure 1-3. Chemical structure of PtOEP

Table 1-2. Luminescence properties of oxygen sensitive dyes immobilized in sol-gel matrixes.

Oxygen sensitive dye	Support matrix	Response time	I_{N_2}/I_{O_2}
Ru(dpp) ₃ 2 ⁺	TEOS sol-gel	0% O ₂ to 100% O ₂ : 3.5s; 100% O ₂ to 0% O ₂ : 30s	12
Ru(dpp) ₃ 2 ⁺	Octyl-triEOS/TEOS sol-gel	None	16.48
Ru(dpp) ₃ 2 ⁺	n-propyl-TriMOS/TFP-TriMOS	Response times were <5s	35
PtTFPP	Octyl-triEOS/TEOS sol-gel	0% O ₂ to 100% O ₂ : 0.6s; 100% O ₂ to 0% O ₂ : 5s	22
PtOEP	Octyl-triEOS/TEOS sol-gel	0% O ₂ to 100% O ₂ : 0.7s; 100% O ₂ to 0% O ₂ : 14s	47
PtTFPP	n-propyl-TriMOS/TFP-TriMOS	0% O ₂ to 100% O ₂ : 3.7s; 100% O ₂ to 0% O ₂ : 5.3s	68.7
PtOEP	n-propyl-TriMOS/TFP-TriMOS	0% O ₂ to 100% O ₂ : 3.7s; 100% O ₂ to 0% O ₂ : 5.9s	82.5

Advantages

Fiber optic sensors have several advantages compared to other measurement techniques. They have rapid response time in the range of 0.2 - 1 s. The main advantage of these probes is they do not consume oxygen but the conventional electrochemical probes do consume oxygen. These are sensitive to low levels of oxygen. Ease of miniaturization and are good for continuous measurements.

Limitations

The main disadvantage of the fiber optic probes is they are sensitive to surrounding light. In order to overcome this overcoating of the fluorophore has been done. However, the overcoating resulted in slow response time, poor reproducibility and fouling. Measurement of DO using these probes is position dependent. A small change

in the position of the fiber or the probe can result in increase or decrease in intensity (counts). Photobleaching of the lumiphore has also been reported.

Table 1-3. Comparison of different methods used to measure DO

Method	Advantages	Disadvantages
Electrochemical probe	Variety of shapes, sizes and consumables. Some probes have build on thermistor for temperature compensation (Lewis 2006)	Oxygen consumption. Electrolyte solution should be free of bubbles to avoid interferences. Membrane fouling, stagnation or rupture.
Optical probe	Ease of miniaturization Do not consume oxygen Sensitive to low levels of oxygen Fast response time: 0.2 – 1 s (Choi and Xiao 2000) Continuous measurements.	Sensitive to ambient light. Photo bleaching of the fluorophore Position dependence of optic fiber
Winkler titration	Official reference method (ASTM D 888-92)	Highly dependent on analyst experience. Contamination with atmospheric O ₂ during titration. Chemical and color interferences
Colorimetric methods	Official methods (ASTM D 5543-94). Kit available. Able to measure ppb of O ₂ .	Not for continuous measurements

Gap in Knowledge

Numerous studies on the effect of dissolved oxygen on the quality of fruit juices and also on the kinetics of dissolved oxygen consumption and ascorbic acid degradation. However, contradictory data is reported on the kinetics of oxygen

consumption and ascorbic acid degradation with kinetic rate as first order, zero order and second order.

There is a need to develop new methods of removing oxygen from juices without removing volatiles and affecting the overall quality of juices. Previous studies on deoxygenation of fruit juices were done by direct addition of enzymes. However, the direct addition of enzymes makes the process uneconomical and in cases violates standards of identity. Enzyme immobilization offers an alternative to this method. To the best of our knowledge, there hasn't been any report about the usage of immobilized GOx-Cat system for deoxygenation in pineapple juice.

Objectives

The specific objectives of this research were

- To better understand the kinetics of dissolved oxygen consumption and ascorbic acid degradation in ascorbic acid solutions and pineapple juices.
- To develop an Immobilized enzyme reactor using GOx-Cat to remove dissolved oxygen from model solutions and fruit juices.

CHAPTER 2 KINETICS OF DISSOLVED OXYGEN CONSUMPTION IN ASCORBIC ACID SOLUTIONS AND PINEAPPLE JUICES

Introduction

Dissolved oxygen plays an important role in the quality of fruit juices. It causes the oxidation of ascorbic acid as well as changes in flavor and color during storage. L-Ascorbic acid (L-AA), degradation occurs by two consecutive or parallel pathways aerobic and anaerobic. During the aerobic pathway, ascorbic acid in aqueous solution is easily oxidized to 2, 3 diketogulonic acid in aqueous solution. Under anaerobic conditions, L-AA is degraded to furfural and rate is slower than aerobic degradation. Various studies on model solutions and citrus juices reported ascorbic acid degradation as first order, zero order and second order kinetics. Numerous studies on the kinetics of dissolved oxygen consumption and ascorbic acid degradation reported contradictory data with kinetic rate as first order, half order and second order.

The oxidation of L-AA to DHA produces color and flavor changes. DHA contains an α -dicarbonyl structure that participates in strecker degradation. Well known examples of strecker aldehydes are methional and acetaldehyde. Oxygen is not always directly responsible for browning in fruit juices. In PPO-catalyzed reactions oxygen is indirectly responsible for browning. The indirect effect is by way of ascorbic acid oxidation to dehydroascorbic acid with further formation of decomposition products such as furfural that has brown color. There is a misassumption that oxygen is always directly responsible for ascorbic acid degradation. L-AA degradation due to DO occurs only during initial stages of storage period from hours to days depending on the storage temperature, light intensity and package permeability. Correlation of rates of DO consumption and L-AA degradation in pure solutions with those of fruit juices help in

better understanding of AA retention in complex juice matrix. Initial studies on oxygen uptake in ascorbic acid solutions described the rate of reaction as first order but there is not agreement among reports in the literature when ascorbic acid concentration is also considered. The objective of this study was to better understand the kinetics of dissolved oxygen consumption and ascorbic acid degradation in ascorbic acid solutions and pineapple juices.

Materials and Methods

Pineapple variety Del Monte Gold extra sweet from Costa Rica was purchased from the local grocery store. The extraction of the pineapple juice was accomplished using a Norwalk juice press (La Jolla, CA). Immediately after the extraction the juice is filled in sterile amber vials for further analysis of dissolved oxygen and ascorbic acid.

Ascorbic acid solutions, 2.8 mM and 28 mM were prepared in deionized, ultra filtered water. The concentration 2.8 mM was chosen to replicate the concentration in orange and pineapple juices. When the probe has an overcoat we observed a slow response so initially we chose high concentration (28 mM) of AA. Probe over coating is explained in Chapter-1. Samples both AA solutions and fruit juices were kept in sterile amber vials without headspace for a period of up to two weeks in temperature-controlled water baths or incubators at 5.0, 13.0, 21.5, 30.5 or 40.0 ± 0.1 °C. Dissolved oxygen and ascorbic acid concentrations were measured at selected time intervals. In order to ensure sterility, all experiments were done in autoclaved/sterilized glass amber vials. Glass amber vials were chosen to protect the sample from light that can accelerate oxidative reactions as suggested by (Mack and others 1976). All the juice extraction equipment, beakers and filter cloth were autoclaved to ensure sterility.

Immediately after extraction the juice was filled in autoclaved amber glass vials under laminar hood.

DO Measurement

DO measurement was performed using a fiber optic FOSPOR oxygen probe without overcoating connected to fiber optic spectrometer model SD-2000 from Ocean Optics, (Dunedin, FL). Because the probe does not have an overcoat samples were protected from surrounding light during the measurement of DO to prevent interferences of light with fluorescence.



Figure 2-1. Dissolved oxygen measurement setup.

AA Measurement

Measurement of AA was performed using capillary electrophoresis every two days for a period of two weeks. The CE system model P/ACE MDQ with DAD and the data acquisition and analysis software Karat 32 version 5.0 was from Beckman Coulter (Fullerton, CA). The capillary was bare fused silica from Polymicro Technologies

(Phoenix, AZ) 50 mm internal diameter, 56 cm total length (48 cm to the detector). The running buffer was 35 mM sodium borate with 5% acetonitrile.

Results and Discussion

Kinetics of DO Consumption in 28 mM and 2.8 mM AA Solutions and Pineapple Juices

Half, first and second order kinetics were used to see which kinetic order fits well for the consumption of dissolved oxygen in both model solutions and fruit juices. First order model fit better initial consumption of dissolved oxygen in ascorbic acid solutions as shown in Figures 2-2, 2-3 and 2-4 and pineapple juices ($R^2 = 0.999$ for first-order, $R^2 = 0.95$ for second-order and $R^2 = 0.85$ for half-order at 21.5°C). Mack and others (1976) also reported that kinetics of dissolved consumption in liquid foods as first order during the first 24h of storage.

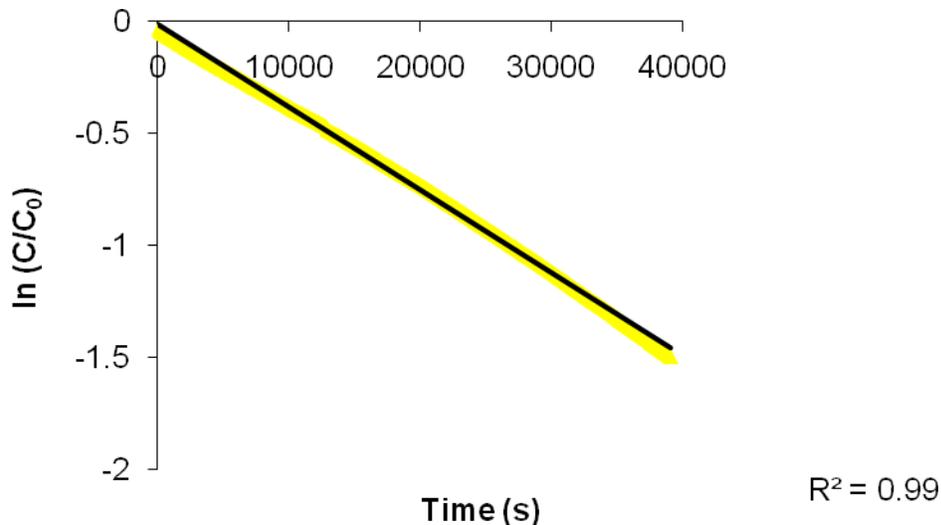


Figure 2-2. First-order kinetics of 2.8 mM AA solutions at 21.5°C.

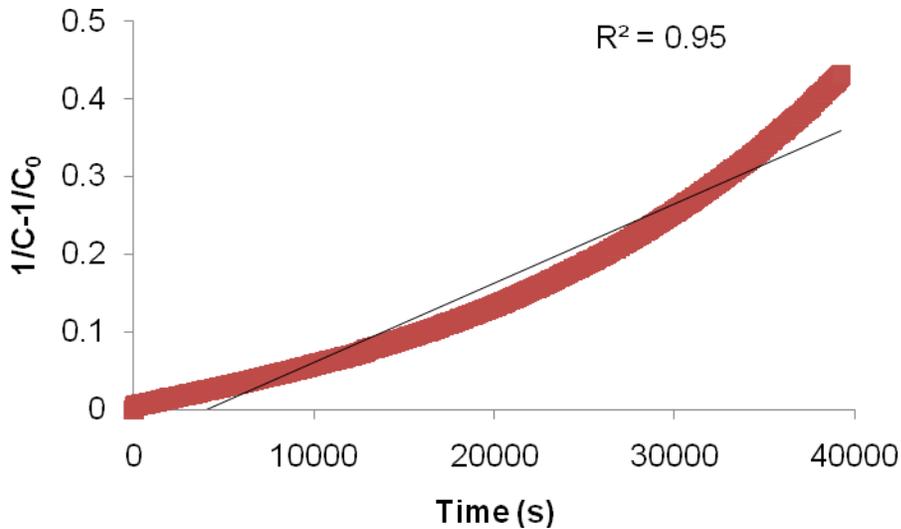


Figure 2-3. Second-order kinetics of 2.8 mM AA solutions at 21.5°C

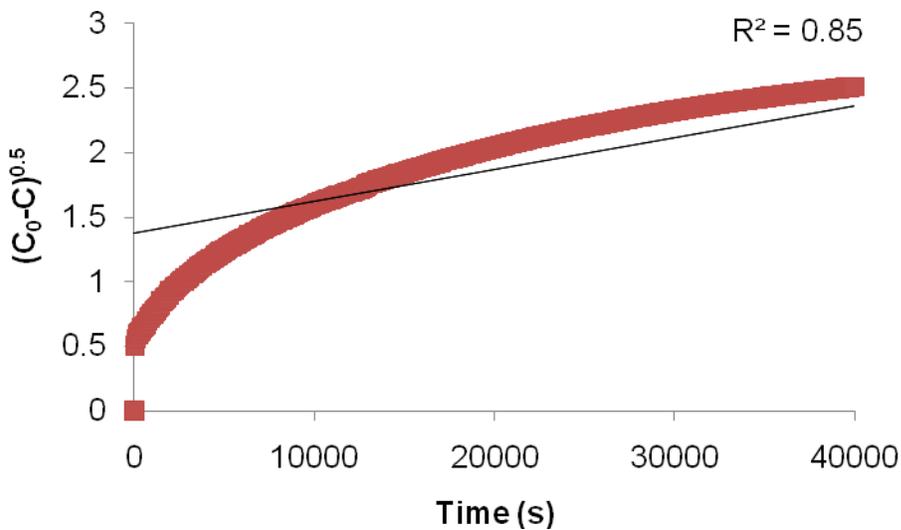


Figure 2-4. Half-order kinetics of 2.8 mM AA solutions at 21.5°C

First-order reaction rate constants ranged between 1.03×10^{-5} to $2.97 \times 10^{-4} \text{ s}^{-1}$ for 5°C and 40 °C, respectively for 28 mM AA solutions and 9.58×10^{-6} to $1.08 \times 10^{-4} \text{ s}^{-1}$ for 13°C and 40 °C, respectively for 2.8 mM AA solutions and between 8.89×10^{-6} to $4.75 \times 10^{-5} \text{ s}^{-1}$ for 13°C and 40 °C, respectively for pineapple juices as shown in Table 2-1 and Table 2-2. The rate constants for 28 mM AA solution are slightly higher

compared to 2.8 mM AA solution. The concentration of Ascorbic acid is almost 500 times and 50 times greater than oxygen concentration in 28 and 2.8 mM AA solutions. Higher concentration were used to obtain faster kinetic data and to ensure that we were kinetically in excess of one the substrates and therefore, we are truly measuring the kinetics of dissolved oxygen and not the kinetics of AA. The rates were higher for 2.8 mM AA solutions compared to pineapple juices. The types of reactions involved in fruit juices other than ascorbic acid degradation are difficult to predict without additional investigations.

The influence of temperature on the first order kinetics of AA solutions and pineapple juices is explained by Arrhenius equation. The first order rate constant of dissolved oxygen consumption in 2.8 mM AA solutions, 28 mM AA solutions and pineapple juices increased exponentially with temperature following Arrhenius behavior (Figure 2-5, Figure 2-7 and Figure 2-9). The activation energy (E_a) was calculated for both AA solutions and pineapple juices. This was determined with linear regression of the logarithm of the rate constant vs. the reciprocal of temperature (Figure 2-6, Figure 2-8 and Figure 2-10). The calculated activation energy values are 67.7 ± 4.2 , 67.12 ± 6.72 and 48.62 ± 2.6 $\text{kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ for 28 mM, 2.8 mM AA solutions and pineapple juices respectively. The decrease in activation energy for pineapple juice compared to AA solutions suggests that the pineapple juice is less sensitive to temperature compared to AA solutions. Mack and others (1976) reported activation energy in liquid infant formula foods at temperature range of 7.2 to 23.9°C as 12.42 to 47.76 $\text{kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$.

Dhuique-Mayer and others (2007) reported ascorbic acid degradation fitted well with first order model in orange and tangerine juice mixture and they calculated the

activation energy as $35.9 \text{ kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ at temperature range of 50 to 100°C . Ahrne and others (1997) reported activation energy of liquid packed food during storage of up to 5 months at 4, 8, 20, 30, 40 and 50°C as $46 \text{ kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$. The model was applied to orange juice aseptically packaged in Tetra Brik Aseptic cartons. Manso and others (2001a) reported activation energy of SSOJ during storage in the absence of light at temperature range of 20 to 45°C as $38.6 \text{ kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$. Garcia-Torres and others (2009) reviewed different studies about ascorbic acid degradation in model solutions and citrus juices. 8 of the 17 studies they reviewed reported L-AA oxidation as first order reaction under different initial DO concentrations. E_a ranged from 35.9 to $143.5 \text{ kJ} \cdot \text{mol}^{-1}$ over a temperature range of 20 and 124°C .

Table 2-1. First order rate constants for dissolved oxygen (DO) consumption in 28 mM and 2.8 mM ascorbic acid solutions, Error is reported as \pm standard deviation

Temp (K)	$k_{\text{DO}} (\text{s}^{-1})$ 28 mM	$k_{\text{DO}} (\text{s}^{-1})$ 2.8 mM
278.15	$1.03 \times 10^{-5} \pm 5.7 \times 10^{-8}$	
286.15	$2.26 \times 10^{-5} \pm 1.6 \times 10^{-7}$	$9.58 \times 10^{-6} \pm 2.76 \times 10^{-6}$
294.65	$6.17 \times 10^{-5} \pm 8.7 \times 10^{-7}$	$2.90 \times 10^{-5} \pm 8.27 \times 10^{-6}$
303.65	$9.75 \times 10^{-5} \pm 1.2 \times 10^{-6}$	$6.71 \times 10^{-5} \pm 5.66 \times 10^{-6}$
313.15	$2.97 \times 10^{-4} \pm 3.21 \times 10^{-6}$	$1.08 \times 10^{-4} \pm 1.05 \times 10^{-5}$

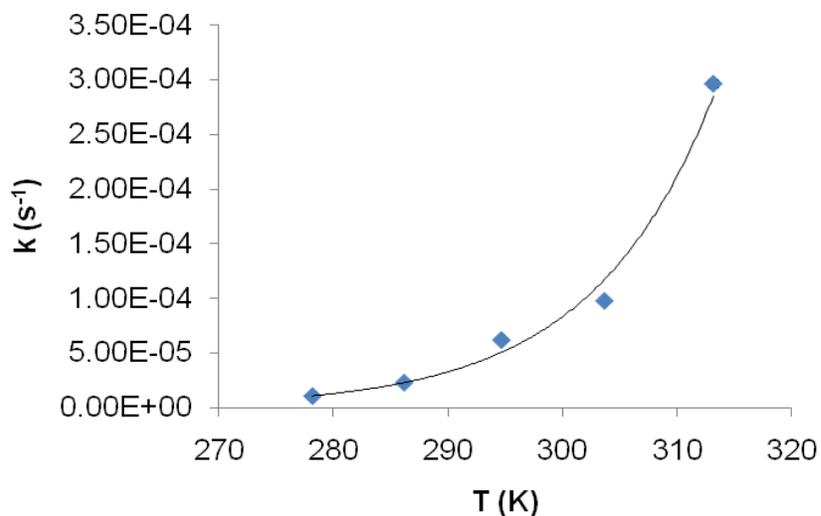


Figure 2-5. Effect of temperature on first order rate constants of dissolved oxygen consumption in 28 mM ascorbic acid solutions, Error is reported as \pm standard deviation.

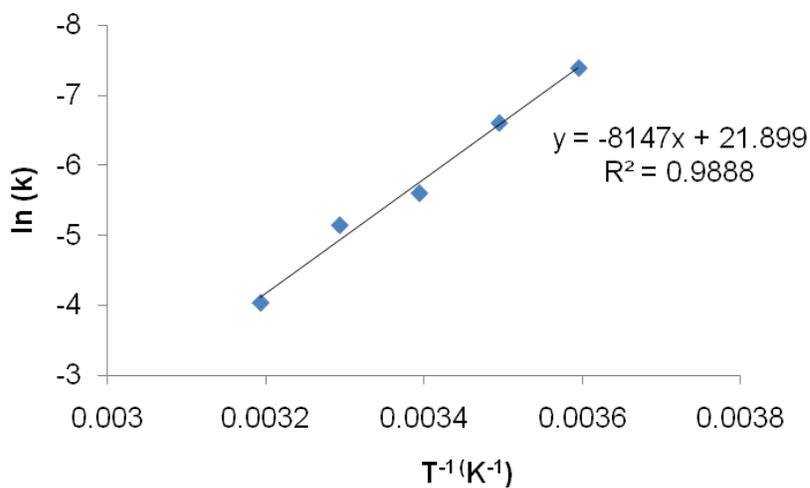


Figure 2-6. Arrhenius plot for rate constant of dissolved oxygen consumption in 28 mM ascorbic acid solutions

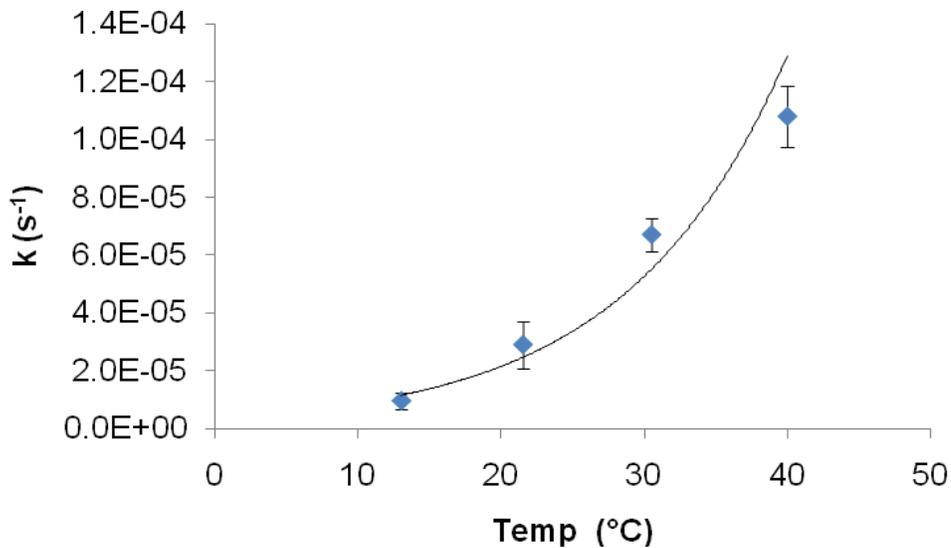


Figure 2-7. Effect of temperature on first order rate constants of dissolved oxygen consumption in 2.8 mM ascorbic acid solutions, Error is reported as \pm standard deviation.

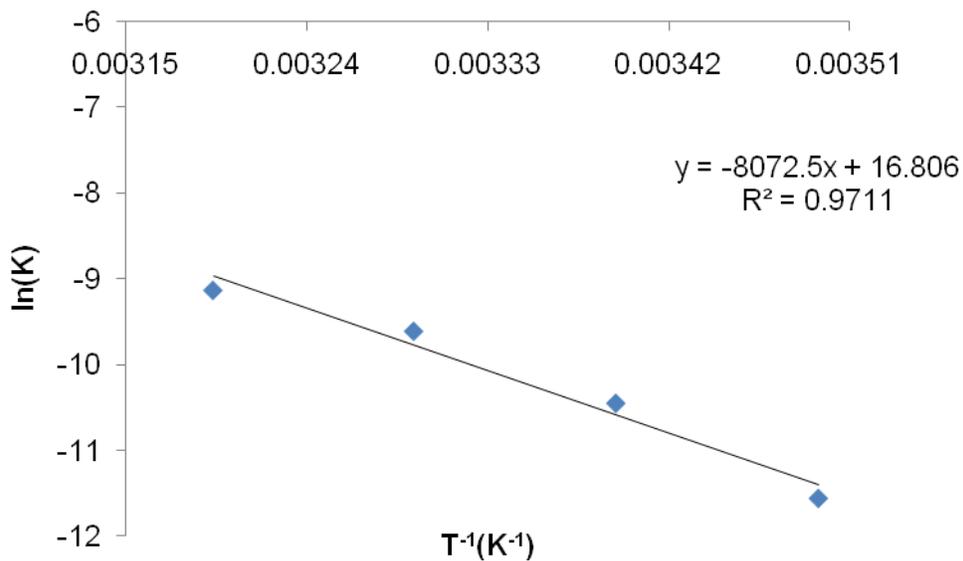


Figure 2-8. Arrhenius plot for rate constant of dissolved oxygen consumption in 2.8 mM ascorbic acid solutions

Table 2-2. First order rate constants for dissolved oxygen (DO) consumption in pineapple juices, Error is reported as \pm standard deviation.

Temp (K)	k_{DO} (s^{-1})
286.15	$8.89 \times 10^{-6} \pm 6.82 \times 10^{-7}$
294.65	$1.30 \times 10^{-5} \pm 2.96 \times 10^{-6}$
303.65	3.11×10^{-5}
313.15	$4.75 \times 10^{-5} \pm 7.25 \times 10^{-6}$

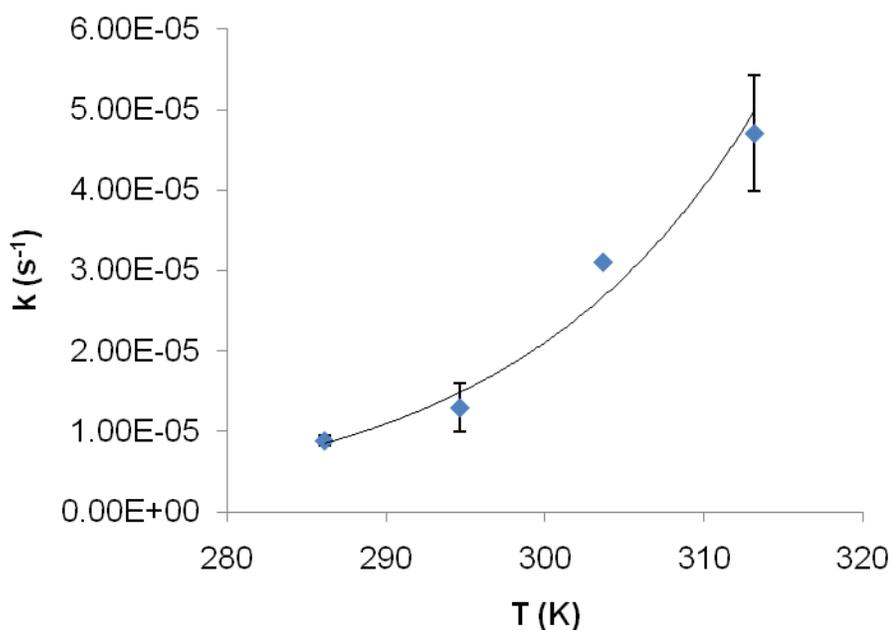


Figure 2-9. Effect of temperature on first order rate constants of dissolved oxygen consumption in pineapple juices, Error is reported as \pm standard deviation.

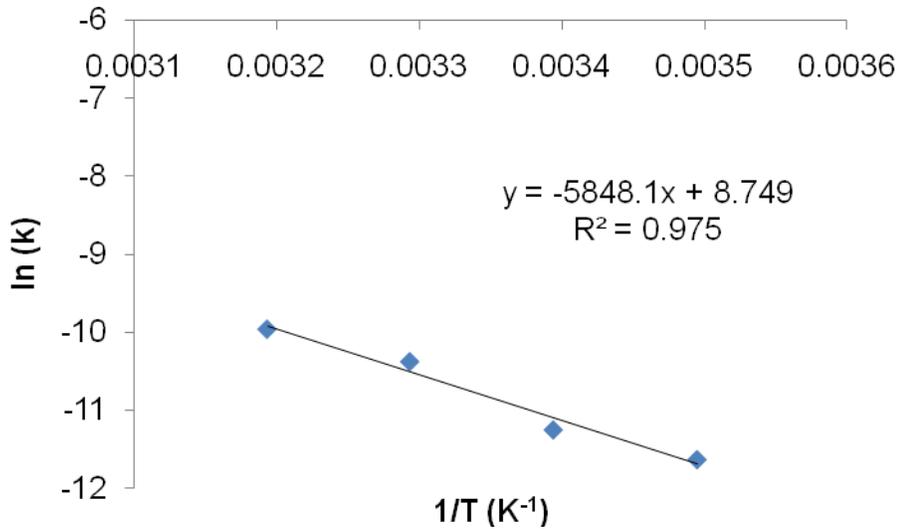


Figure 2-10. Arrhenius plot for rate constant of dissolved oxygen consumption in pineapple juices

Kinetics of AA in 2.8 mM AA Solutions and Pineapple Juices

Measurement of AA was performed every two days in both 0.05% (2.8 mM) AA solutions and pineapple juices. In 2.8 mM AA solutions there is a loss of 23.62% and 25.28% AA was observed at 13°C and 21.5°C after 10 days storage period. There is a 18.7% and 25.2% decrease in AA found after 8 days of storage at 30.5°C and 40°C. We did not observe any significant difference in decrease in Ascorbic acid between 30.5°C and 40°C after 8 days of storage.

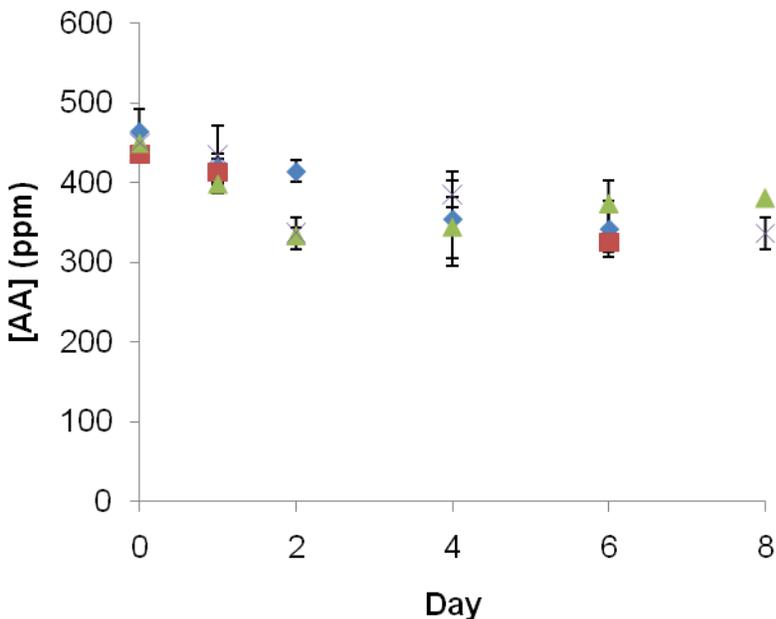


Figure 2-11. Concentration of AA in 0.05% AA solutions at four different temperatures (\blacklozenge 13 °C, \blacksquare 21.5 °C, \blacktriangle 30.5 °C, \blacktimes 40 °C), Error is reported as \pm standard deviation.

Measurement of Ascorbic acid was done at four different temperatures for every two days for a period of 10 days. AA concentration decreased by 27.9% at 13°C after 10 days, 33.5% and 31.9% decrease after 6 days of storage at 30.5°C and 40°C respectively. We would expect temperature to have significant impact on the ascorbic acid degradation. Although AA concentration decreased at all temperatures, temperature did not show a significant difference in decrease in the rate of AA degradation at 30.5°C and 40°C after 6 days of storage. These results suggest that the activation energy is very small. Therefore, despite reporting rate constants at four different temperatures, our experimental results do not allow reasonable calculation of the activation energy. Also, in view of the much faster reaction of oxygen, it is evident that AA degradation in this experiment was anaerobic. Longer experiments and wider temperature ranges need to be studied to accurately determine activation energy.

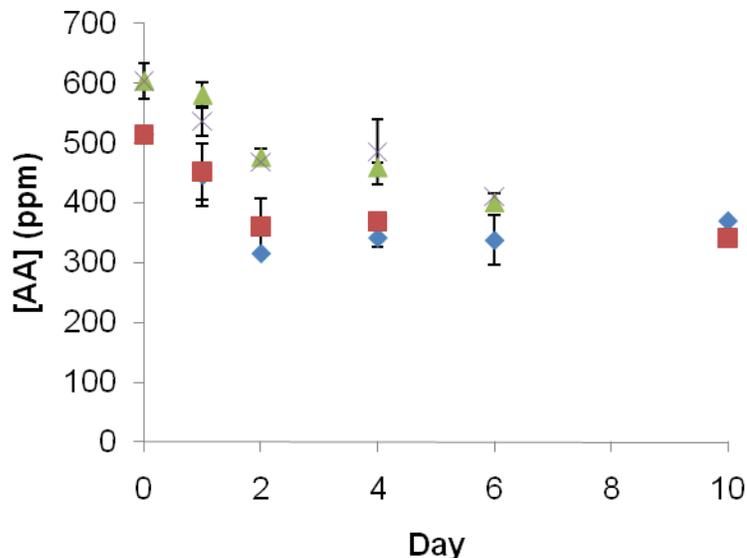


Figure 2-12. Concentration of AA in pineapple juices at four different temperatures (♦13 °C, ■21.5 °C, ▲30.5 °C, ×40 °C), Error is reported as \pm standard deviation.

Conclusions

Kinetics of dissolved oxygen consumption fitted well with first order kinetics in both AA solutions and pineapple juices. The first order rate constants for DO consumption for 28 mM AA solutions are slightly higher compared to 2.8 mM AA solutions. The first order rate constants for DO consumption in 0.05% AA solutions are higher than pineapple juices at all the four temperatures. Rate of DO consumption in juice matrix is slower than aqueous ascorbic acid solutions. This suggests that the several other reactions occur simultaneously in fruit juices. The types of reactions involved in fruit juices other than ascorbic acid degradation are difficult to predict without additional investigations. The kinetics of dissolved oxygen consumption as a function of temperature followed Arrhenius relationship. The activation energies of dissolved oxygen consumption are 67.7 ± 4.2 , 67.12 ± 6.72 and 48.62 ± 2.6 $\text{kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ for 28 mM, 2.8 mM AA solutions and pineapple juice respectively. There is no significant difference between 28 mM and 2.8 mM AA solutions. However, lower activation energy

for pineapple juice compared to AA solutions was observed. This suggests that the pineapple juice is less sensitive to temperature compared to AA solutions. Temperature did not show a significant difference in decrease in the rate of AA degradation between 13°C and 40°C. Longer storage times and wider temperature ranges might help in observing temperature effects and calculating activation energy.

CHAPTER 3 THIN FILM ENZYME REACTOR FOR DEOXYGENATION OF MODEL SOLUTIONS AND PINEAPPLE JUICE

Introduction

Dissolved oxygen (DO) plays an important role in the quality of juices. It accelerates oxidation of vitamin C and causes non-enzymatic browning during storage leading to the formation of off-flavors (Trammell and others 1986; Rassis and Saguy 1995). Degradation of ascorbic acid is one of the major causes of color and quality changes during storage. To prevent these deleterious effects, fruit juices are often deaerated prior to pasteurization. Deoxygenation is done by vacuum flash deaeration, gas sparging, oxygen scavengers, membrane deaerators and adding antioxidants or using enzymes. Vacuum deaeration is the most commonly used method in the citrus industry. However, in this method along with oxygen essential oil is also removed (Braddock 1999). Gas sparging consists of displacing the DO with another gas such as nitrogen or carbon dioxide. Early studies on methods of deaeration indicate that in orange juice 99% of DO was removed using nitrogen sparging compared to 77% DO removed by vacuum deaeration (Joslyn 1961) but significant amount of volatiles are also removed in this process. Jordan and others (2003a) reported that the greatest losses in concentration of volatiles occurred during industrial deaeration. Enzymatic deaeration of juices using GOx-Cat in solution has been reported by (Sagi and Mannheim 1990; Parpinello and others 2002) in orange juice, apple and pear purees. The advantage of this system is glucose is present in large excess in juices with respect to DO so there is no need to add any substrate. Detailed literature review on enzymatic deaeration and other deaeration methods was explained in Chapter 1.

However, the direct addition of enzymes makes the process uneconomical and in cases violates standards of identity. Enzyme immobilization offers an alternative to this method. Enzyme immobilization can be done by adsorption, covalent bonding, encapsulation, entrapment in gels, cross-linking, hydrophobic interaction and polymer entrapment. Unlike conventional immobilization methods, the electrochemical formation of polymer layers of controlled thickness gives reproducible and non manual procedure for sensor fabrication. To the best of our knowledge, there hasn't been any report about the usage of immobilized GOx-Cat system for deoxygenation in pineapple juice.

The objective of this study was to develop and test a laboratory prototype deaeration reactor immobilizing GOx-Cat in electrochemically generated poly-*o*-phenylenediamine thin films deposited on the interior wall of a platinized platinum tubes.

Materials and Methods

Glucose oxidase (EC 1.1.3.4 type X-S from *Aspergillus niger*), Catalase (EC: 1.11.1.6), *o*-phenylenediamine free base, chloroplatinic acid hexahydrate, lead acetate trihydrate, hydrogen peroxide and potassium chloride were purchased from Sigma-Aldrich Chemical Co. (St.Louis, MO) Platinum wire (0.4mm diameter) was purchased from Fischer Scientific. Pt metal tube 250 mm length (1.60 mm o.d, 1.30mm i.d) was purchased from American Elements, (Los Angeles, CA). All other reagents and solvents were reagent grade. Solutions were prepared in deionized ultrafiltered water.

Platinization, electro polymerization and cyclic voltammetry experiments were performed with a potentiostat/galvanostat, EG&G 263A (Perkin-Elmer Instruments, Oak Ridge, TN), interfaced to a personal computer, through a GPIB board (National Instruments, Austin, TX).

All potentials are reported with respect to Ag/AgCl, 3.0 M KCl reference electrode model EE008 from cypress systems inc. (Lawrence, KS) A Pt wire was used as the counter electrode. A syringe pump model 210 from Kd Scientific (New Hope, PA) was used for the injection of model solutions and juices. The syringe pump was interfaced to and controlled with a PC through a serial RS-232 port.

Reactor Cleaning

Reactor was polished with 5- μm alumina slurry (Buehler, Lake Bluff, IL) using miniature brushes purchased from Tanis Inc. (Waukesha, WI), rinsed with deionized water and immersed in an ultrasound bath for 5 min to remove any adherent alumina particle. For reuse, prior to polishing with alumina and after sonication, Pt tubes were immersed in concentrated H_2SO_4 at 21°C for at least 1 h and rinsed with deionized water.

Reactor Platinization

Platinization was carried at -100 mV vs Ag/AgCl in 4 mM H_2PtCl_6 , 1 mM $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)$ (used as crystal growth promoter), 0.1 M KCl oxygen-free solution for 60 min at room temperature 21°C (Reyes-De-Corcuera and others 2005). Solutions were deoxygenated by vigorously sparging with ultrapure nitrogen gas. A Pt wire inserted in the capsule was used as the counter electrode. To prevent counter electrode from touching the working electrode, thin glass beads were fused on the wire. Platinization of the internal wall of the reactor was performed to increase the surface area.

Reactor Polymerization

Potentiostatic electropolymerization was carried out in 5 mM *o*-phenylenediamine, 0.2 M acetate buffer (pH 5.2) and 10 g L⁻¹, 25 g L⁻¹ and 50 g L⁻¹ GOx solutions at 650 mV vs. Ag/AgCl for 30 min at 21°C. Before applying the polymerization potential,

monomer and enzyme were allowed to diffuse for 5 min into the porous pt deposit. The concentration of catalase was adjusted from 45 μL to 112.5 μL to ensure complete decomposition of H_2O_2 . The reactors were stored in 0.1 M phosphate buffer pH 7.0 until further testing.

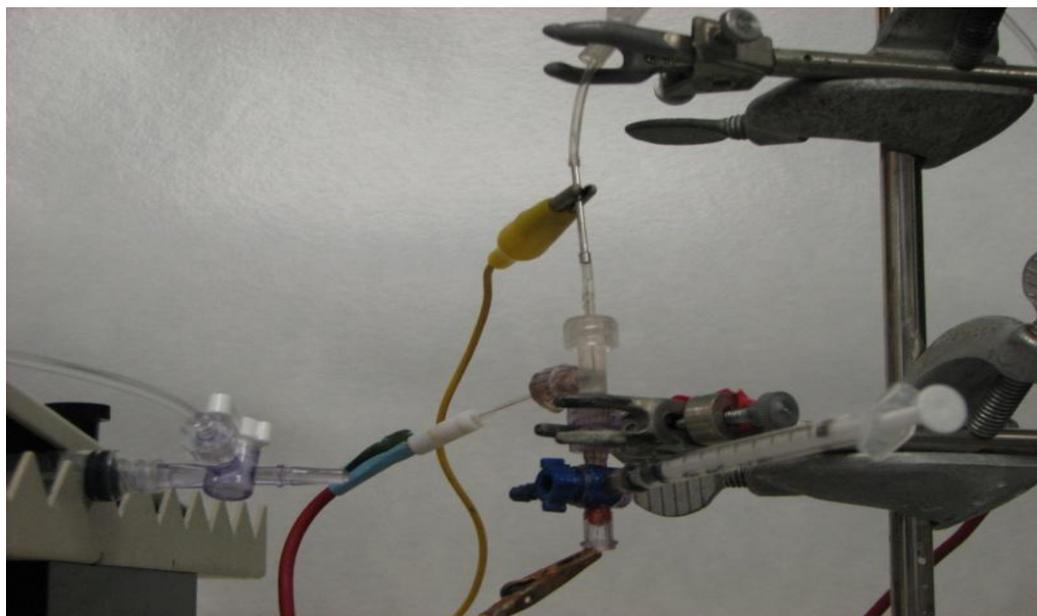


Figure 3-1. Enzyme immobilization setup.

Model Orange Juice Solution

A model orange juice solution was prepared 2.5% glucose, 2.5% fructose, 5.0% sucrose, 0.8% citric acid in distilled water (Peleg and others 1992). The pH of the above solution was adjusted to 3.5, 4.7, 5.9, 7.0 using 4 N NaOH for further testing.

Pineapple Juice Preparation

Pineapple variety Del Monte Gold “extra sweet” from Costa Rica was purchased from the local grocery store. The extraction of the pineapple juice was done using a Norwalk juice press (La Jolla, CA). Soluble solids were measured using Leica Auto ABBE refractometer. Both acidity and pH of the juice was measured using Schott Titroline easy automatic titrator.

Testing the Reactor

Initial tests with the reactors were carried out using 166 mM glucose solutions in 0.1 M phosphate buffer pH 7.0. This concentration was chosen to replicate the D-glucose concentration in orange juice (3.0%). 166 mM glucose solutions, model orange juice solutions and pineapple juice were pumped using a syringe pump at three different flow rates 0.075, 0.05 and 0.025 mL min⁻¹. Operation parameters enzyme concentration, length and flow rate were adjusted to remove the highest % of DO.

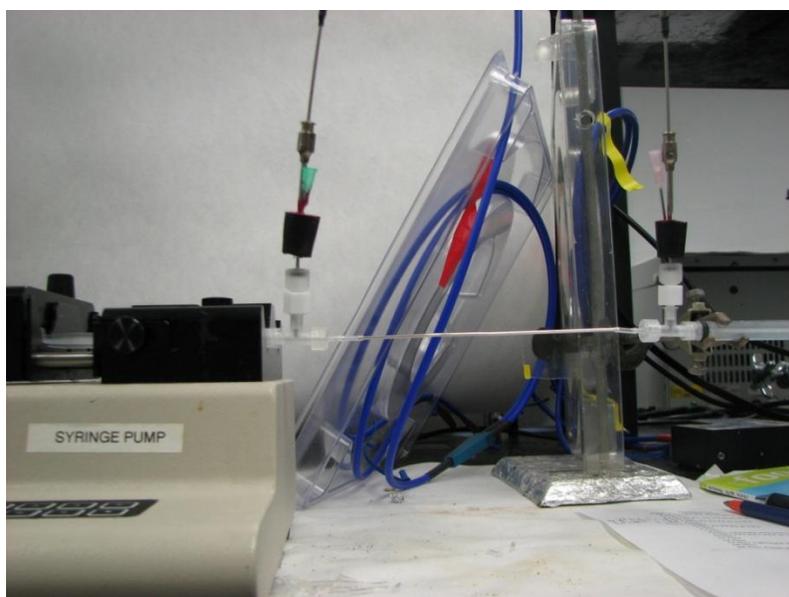


Figure 3-2. Setup for testing the reactor

Electrochemical determination of current was done using potentiostat at 700 mV. The current is a measure of the rate of production of H₂O₂ by the enzymatic reaction which is the principle of operation of amperometric glucose biosensors (Reyes-De-Corcuera and others 2005) .

Measurement of Dissolved Oxygen

DO measurement was done using a fiber optic FOSPOR oxygen probe without over coating connected to USB 4000 spectrometer from Ocean Optics, (Dunedin, FL).

Calibration of the probe is the important step in the measurement of DO and was done using Stern-Volmer equation (Chapter 2). In the model solutions the standard 0% oxygen concentration was obtained by using 5% sodium sulfite solution in water in five minutes whereas in the pineapple juice the 0% concentration was achieved using 3% Glucose oxidase in 10 minutes. The other standard used was air saturated sample. The concentration of air saturated sample is dependent on temperature and pressure. At 22 °C and at a pressure of 101.325 kPa the concentration of dissolved oxygen in saturated water sample is 275 μM or 8.8 ppm. The Stern-Volmer constant is temperature dependent so all the measurements are done at constant temperature. Because the probe did not have an overcoat samples were protected from light during the measurement of DO to prevent interferences of light with fluorescence. Initially we used the probe with overcoating to avoid interferences from environmental light. However, due to slow response time, poor reproducibility and fouling we switched back to probes without overcoating. Detailed review on measurement of DO was presented in Chapter 1.

Results and Discussion

Effect of Enzyme Concentration

The amount of dissolved oxygen removed increased with the enzyme concentration used in the immobilization solution as shown in Table 3-1. Reactors 3-cm long with 10 g L⁻¹ GOx removed 41.1% DO. In contrast, reactors 3-cm long with 25 g L⁻¹ and 50 g L⁻¹ GOx removed 87.7% and 86.4% of DO respectively. There was no significant difference in DO removal by reactors with 25 g L⁻¹ and 50 g L⁻¹ which is most probably due to substrate diffusion becoming limiting or electrochemically generated films with higher GOx concentration have reduced permeability and increased catalytic

activity. Extended operation of reactors with high concentration of enzyme lead to film detachment.

Table 3-1. Performance of 3 cm reactors at selected GOx concentrations in model orange juice solution determined as [DO] at the exit of the reactor. Flow rate was $0.025 \text{ mL min}^{-1}$, error is reported as \pm standard deviation

[Enzyme] (g L^{-1})	Initial [DO] (μM)	Final [DO] (μM)	DO removed (%)
10	276.6 ± 27.2	163.5 ± 26.3	41.1 ± 3.74
25	264.7 ± 10.1	32.8 ± 10.9	87.7 ± 3.65
50	271.4 ± 11.6	36.9 ± 6.5	86.5 ± 1.82

Current response also almost doubled as GOx concentration increased from 10 to 25 g L^{-1} in glucose solutions up to 300 mM. However, there was little change in current when increasing [GOx] from 25 g L^{-1} to 50 g L^{-1} as shown in Figure 3-3. This further supports that at higher GOx concentrations the rate of diffusion of substrates and products becomes limiting. No replication was done for each concentration in this experiment but the relative increase in current response between 10 g L^{-1} and 25 g L^{-1} or 50 g L^{-1} is consistent throughout the glucose concentration range.

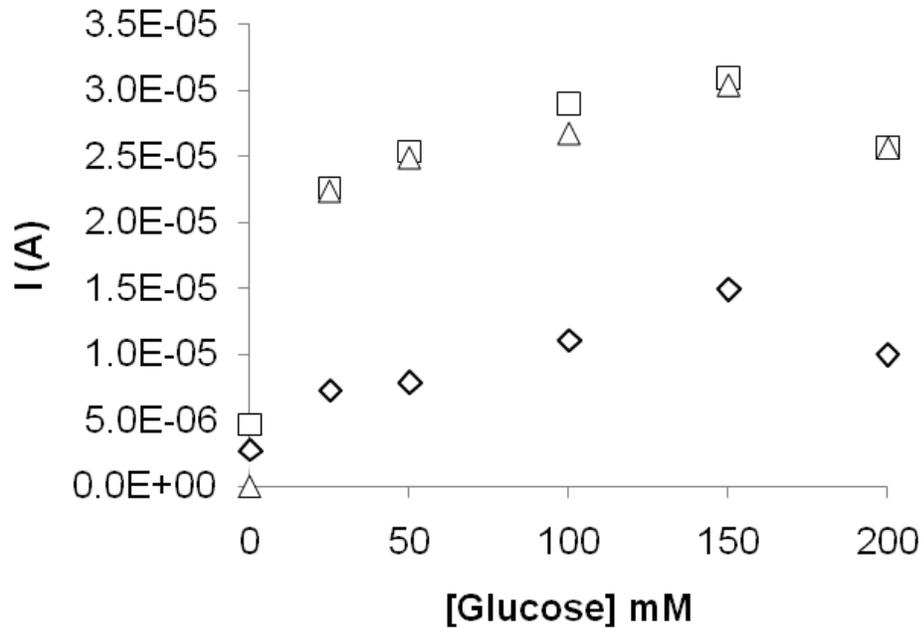


Figure 3-3. Effect of GOx concentration (\diamond 10 g/L, \square 25 g/L, \triangle 50 g/L) and glucose concentration on the amperometric response of 3-cm GOx reactors at a flow rate of 0.5 mL min^{-1} .

Effect of Glucose Concentration

Current response vs. glucose concentration showed a maximum at approximately 75 mM. There is no significant difference in the current response by the reactor at glucose concentrations 75 mM to 200 mM (Figure 3-4). A slight decrease in current response was observed after 75 mM which might be due to substrate inhibition as previously reported for GOx biosensors (Shin and Kim 1995; Reyes-De-Corcuera and others 2005). The concentration of glucose in orange and pineapple juices is around 166mM suggesting that the glucose concentration has little effect on the performance of the reactor.

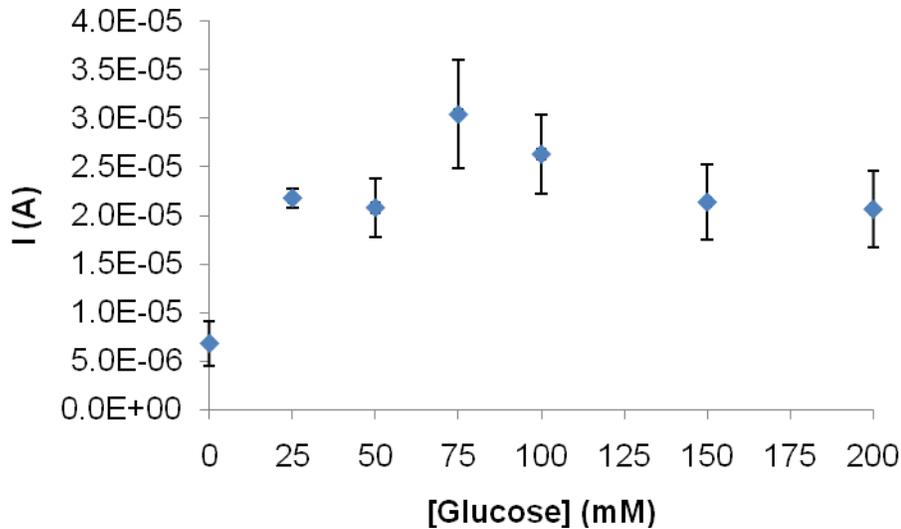


Figure 3-4. Effect of glucose concentration on the amperometric response of a 3-cm, 25 g L⁻¹ GOx reactor at a flow rate of 0.1 mL min⁻¹, Error is reported as ± standard deviation.

Effect of Retention Time

Reactor length

Increasing the length of reactor to 12-cm significantly increased DO removal as indicated in Figure 3-5. However, for 12-cm or 18-cm reactors oxygen consumption leveled off due to the small concentration of DO. The concentration of DO decreased from 270 μM to 21.5 μM using a 12-cm reactor. Commercial storage of deaerated orange juice is typically 15.63 μmol (0.5 ppm) indicating that the prototype reactor is capable of meeting the desired DO concentration.

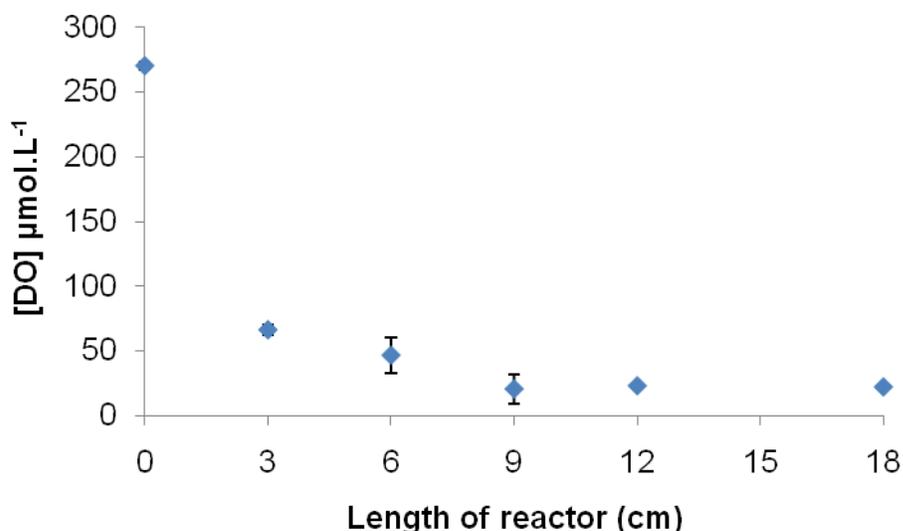


Figure 3-5. Effect of reactor length on the performance of (25 g L⁻¹ GOx- 2X cat) reactors in model orange juice solution at a flow rate of 0.025 mL min⁻¹, Error is reported as ± one standard deviation.

Flow rate

Retention time was also increased by adjusting flow rate for 3-cm, 12-cm long reactors. Retention times of 31.8 s and 95.5 s resulted in 22.6 and 46.3% DO removal respectively for 3-cm reactors. Retention times of 127.4 s and 382.4 s resulted in 63.6 and 91.6% DO removal respectively for 12-cm reactors as indicated in Table-3. With retention time of 382 s, (12-cm reactor at a flow rate of 0.025 mL min⁻¹) 90% removal of DO was achieved which corresponds to 23.1 μM (0.74 ppm). The Reynolds number for the 12 cm reactor at these three different flow rates is below 1. This number is very small and it is difficult to operate at industrial level with these parameters. However, by adjusting retention time, tube length and diameter to a retention time of 382 s it is possible to remove more than 90% of DO. The industrial conditions of 4.54 kg/min could be achieved by stacking the reactors and running them in parallel. Reactors can be stacked to achieve scale up.

Table 3-2. Effect of flow rate on the performance of 25 g L⁻¹ GOx reactor in 166 mM glucose solutions, Error is reported as ± one standard deviation.

Length (cm)	Retention time (s)	DO removed (%)	Flow rate (mL min ⁻¹)	Initial [DO] (µM)	Final [DO] (µM)
3	31.8	22.6 ± 6.7	0.075	279.0 ± 11.3	216.5 ± 27.6
3	63.6	33.1 ± 6.1	0.05	279.5 ± 2.1	187 ± 15.6
3	95.5	46.3 ± 10.3	0.025	284.3 ± 3.8	153 ± 31.2
12	127.4	63.6	0.075	250.0	91.0
12	254.7	76.0	0.05	242.0	58.0
12	382.4	91.6 ± 0.6	0.025	274.0 ± 0.7	23.0 ± 1.4

Effect of pH

The effect of pH on the performance of the reactor was very small as shown in Table 3-3. There was no significant difference between effect of pH on the performance of reactor in the range of 3.5 to 7.0. Glucose oxidase from *Aspergillus niger* has optimum activity at 5.5 to 6.0. From pH 4.0 to 7.0 GOx has at least 90% of its activity (Kalisz and others 1990). Catalase from *Micrococcus* sp., has a working pH of 3 to 9 and the working optimum pH is 5.5. Kothapalli and others (2007) reported that there was no significant difference between GOx immobilized in low-density polyethylene using UV polymerization and free GOx at temperatures 30 , 25 and 10 °C. Previous reports in the literature indicate that GOx-Cat system is dependent on pH. All these reports are based on solution. i.e. direct addition of the enzymes. Mistry and Min (1992) reported that GOx-Cat complex showed a maximum activity at pH 6.0 and 30 °C in model salad dressing. Pickering and others (1998) reported that in Riesling grape using

GOx-Cat concentration of 2 g L⁻¹ the gluconic acid produced at pH 6.0 (80 g L⁻¹) was almost 3 times the gluconic acid produced at pH 3.1 (30 g L⁻¹). Parpinello and others (2002) reported that GOX100 (glucose oxidase-catalase system, Gist-Brocades, Lille, France) showed maximum activity at pH 5.5 and 6.5 corresponding to 50 and 12 units/mg and at pH 4.0 the activity decreased to 15 and 5 units/mg for glucose oxidase and catalase respectively. One unit of glucose oxidase activity refers to 1 μmol of gluconic acid formed per min at 35 °C and pH 5.1. In all the above cases the enzymes were directly added to the samples. Our results with immobilized GOx-Cat differ from these previous reports on soluble enzymes indicating that immobilization widened the pH operational range of GOx-Cat.

Table 3-3. Effect of pH on the performance of 3 cm (GOx-Cat) reactor in model orange juice solutions at a flow rate of 0.025 mL min⁻¹, Error is reported as ± one standard deviation.

pH	Initial [DO] (μM)	Final [DO] (μM)	DO removed (%)
3.5	277.7 ± 16.8	66.2 ± 3.78	75.9
4.7	256.9 ± 12.9	70.0 ± 26.7	72.8
5.9	288.9 ± 20.8	54.1 ± 8.2	81.2
7.0	279.3 ± 12.6	63.1 ± 5.5	77.4

Testing with Model Solutions and Fruit Juices

In both model solutions and pineapple juice approximately 90% DO was removed using a 12-cm reactor with retention time 6.4 min. Sagi and Mannheim (1990) reported that GOx-Cat enzyme preparation of 17 units L⁻¹ reduced oxygen concentration to less than 1 ppm in 30 minutes at room temperature in orange juice. Parpinello and others

(2002) reported that 2 units L⁻¹ of GOx-Cat removed 99% of DO in 120s from apple and pear purees. Ough (1975) reported that GOx-Cat effectively removed DO from white and rose table wines. Hydrogen peroxide was reduced by sulfur dioxide rather than catalase. In all the above cases the enzyme complex was directly added to the samples which increases process operation costs and it is not feasible for orange juice NFC because it would violate the standards of identity.

Table 3-4. Performance of 12 cm (25 g L⁻¹ GOx- 2x Cat) reactor with model orange juice solution, glucose solution and pineapple juice, Error is reported as ± one standard deviation

Solution	Initial [DO] (μM)	Final [DO] (μM)	DO removed (%)	Acidity (%)	pH
150 mM glucose	265.0	21.5	91.9	0.0	7.0
Model orange juice	274.7 ± 1.2	29.6 ± 16.6	89.2 ± 6.0	0.8	3.5
Pineapple juice	281.3 ± 7.8	23.5 ± 2.5	91.7 ± 0.7	0.59 ± 0.03	3.0

GOx-Cat immobilized in poly-o-phenylenediamine successfully removed more than 90% DO in both model solutions and fruit juices.

Conclusions

Reactors with 10 g L⁻¹ and 25 g L⁻¹ GOx showed better operational stability compared to 50 g L⁻¹ where film detachment was observed. Increasing the retention time from 127.4 s to 382.4 s has increased the DO removal from 63.6% to 91.6% in a 12-cm reactor. Varying the pH from 3.5 to 7.0 had very little impact on the performance of reactor. In both model solutions and pineapple juice approximately 90% DO was

removed using a 12-cm reactor at a flow rate of $0.025 \text{ mL min}^{-1}$ with a retention time of 6.4 min.

Over all Conclusions

Kinetics of dissolved oxygen consumption fitted well with first order kinetics in both AA solutions and pineapple juices. The initial rate of DO consumption is faster in AA solutions compared to pineapple juices. The kinetics of DO consumption as a function of temperature followed Arrhenius relationship. The activation energies of dissolved oxygen consumption are 67.7 ± 4.2 , 67.12 ± 6.72 and $48.62 \pm 2.6 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ for 28 mM, 2.8 mM AA solutions and pineapple juices respectively. Temperature did not show a significant difference in decrease in the rate of AA degradation between 13°C and 40°C . Longer storage studies might help in determining effect of temperature and activation energy calculation.

Reactors with 25 g L^{-1} GOx showed better operational stability and performance compared to 10 and 50 g L^{-1} . Although the enzyme activity depends on pH we did not observe any effect of pH on the performance of reactor. In principle, we would expect the percentage of oxygen removed to be half compared with no catalase. But, we did not observe any effect of catalase (2 times GOx concentration) on the performance of reactor. However, when increasing the catalase concentration from 2-times- 5-times the activity of GOx we did not observe any decrease in oxygen concentration. GOx-Cat immobilized enzyme reactor removed more than 90% of DO in both model solutions and pineapple juices with a retention time of 6.4 min.

Future Work

With the current FOSPOR or FOXY probe we were not able to reproducibly measure DO in orange juice as these probes degraded in that juice. Similar study with

both DO consumption (with a different type of sensor) and ascorbic acid degradation in orange juice would help in better understanding of AA behavior in real juice as orange is very rich in AA.

The current enzymatic reactor is successful in removing more than 90% of DO in both model solutions and pineapple juices. Further analysis has to be done on the quality parameters of enzymatically deoxygenated juice (flavor, color). Immobilization of enzymes in electrochemically generated polymer thin films resulted in stable and reproducible formation of layers. This is one of the most successful methods in the preparation of biosensors. Development of thick and stable films using other polymers and immobilization methods might help in improving the operational performance and stability of the reactor. We were not able to detect the effect of catalase concentration on the performance of reactor. There is also a need to better understand the spatial arrangement of enzyme molecules especially when a high concentration of catalase is used as this would help in successful immobilization of both GOx and catalase.

LIST OF REFERENCES

- Agullo E, Rodriguez MS, Ramos V, Albertengo L. 2003. Present and future role of chitin and chitosan in food. *Macromolecular Bioscience* 3(10):521-30.
- Ahrne LM, Oliveira FAR, Manso MC, Drumond MC, Oste R, Gekas V. 1997. Modelling of dissolved oxygen concentration during storage of packaged liquid foods. *Journal of Food Engineering* 34(2):213-24.
- Baiano A, Marchitelli V, Tamagnone P, Nobile MA. 2004. Use of active packaging for increasing ascorbic acid retention in food beverages. *JFS* 69(9):E502 - E8.
- Baticz O, Tomoskozi S. 2002. Determination of total cholesterol content in food by flow injection analysis with immobilized cholesterol oxidase enzyme reactor. *Nahrung-Food* 46(1):46-50.
- Braddock R. 1999. *Handbook of Citrus by-products and processing technology*. New York: John Wiley & sons
- Campbell A, Uttamchandani D. 2004. Optical dissolved oxygen lifetime sensor based on sol-gel immobilisation. *IEEE Proceedings-Science Measurement and Technology* 151(4):291-7.
- Castellari M, Matricardi L, Arfelli G, Carpi G, Galassi S. 2000. Effects of high hydrostatic pressure processing and of glucose oxidase-catalase addition on the color stability and sensorial score of grape juice. *Food Science and Technology International* 6(1):17-23.
- Choi MMF, Xiao D. 2000. Single standard calibration for an optical oxygen sensor based on luminescence quenching of a ruthenium complex. *Analytica Chimica Acta* 403:57-65.
- Chu CS, Lo YL. 2007. High-performance fiber-optic oxygen sensors based on fluorinated xerogels doped with Pt(II) complexes. *Sensors and Actuators B-Chemical* 124(2):376-82.
- Cole CA, Genetell EJ. 1970. Decarbonation and deaeration of water by use of selective hollow fibers. *Environmental Science & Technology* 4(6):514-&.
- Coughlin RW, Charles M, Julkowski K. 1978. Experimental results from a pilot plant for converting acid whey to potentially useful food products. *AIChE Symposium Series* 74(172):40-6.
- Dhuique-Mayer C, Tbatou M, Carail M, Caris-Veyrat C, Dornier M, Amiot MJ. 2007. Thermal degradation of antioxidant micronutrients in Citrus juice: Kinetics and newly formed compounds. *Journal of Agricultural and Food Chemistry* 55(10):4209-16.

- Falade KO, Babalola SO, Akinyemi SOS, Ogunlade AA. 2004. Degradation of quality attributes of sweetened Julie and Ogbomoso mango juices during storage. *European Food Research and Technology* 218(5):456-9.
- Garcia-Torres R, Ponagandla NR, Rouseff RL, Goodrich-Schneider RM, Reyes-de-Corcuera JI. 2009. Effects of dissolved oxygen in fruit juices and methods of removal. *Comprehensive Reviews in Food Science and Food Safety* 8(4):409-23.
- Gill CB, Menneer ID. 1997. Advances in gas control technology in the brewery. *The Brewer*:76-84.
- Grosova Z, Rosenberg M, Rebros M. 2008. Perspectives and applications of immobilised beta-galactosidase in food industry - a review. *Czech Journal of Food Sciences* 26(1):1-14.
- Illanes A, Wilson L, Raiman L. 1999. Design of immobilized enzyme reactors for the continuous production of fructose syrup from whey permeate. *Bioprocess Engineering* 21(6):509-15.
- Jiahui Shao, Huifeng Liu, Yiliang He. 2008. Boiler feed water deoxygenation using hollow fiber membrane contactor. *Desalination* 234 (1-3):370-77
- Jiang DS, Chen X, Liu E, Huang J. 2002. Preparation and properties of sensing membrane for fiber optic oxygen sensor. *Journal of Wuhan University of Technology-Materials Science Edition* 17(2):51-3.
- Johnson JR, Braddock RJ, Chen CS. 1995. Kinetics of ascorbic acid loss and nonenzymatic browning in orange juice serum: Experimental rate constants. *JFS* 60(3):502-5.
- Jordan MJ, Goodner KL, Laencina J. 2003a. Deaeration and pasteurization effects on the orange juice aromatic fraction. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology* 36(4):391-6.
- Jordan MJ, Goodnerb KL, Laencinaa J. 2003b. Deaeration and pasteurization effects on the orange juice aromatic fraction. *Lebensm-Wiss U-Technol* 36:391-6.
- Joslyn M. 1961. Physiological and enzymological aspects of juice production. DK T, editor. Westport: Conn: Avi Pub Co
- Kalisz HM, Hecht HJ, Schomburg D, Schmid RD. 1990. Crystallization and preliminary-x-ray diffraction studies of a deglycosylated glucose-oxidase from *aspergillus-niger*. *Journal of Molecular Biology* 213(2):207-9.
- Kennedy JF, Rivera ZS, Lloyd LL, Warner FP, Jumel K. 1992. L-ascorbic acid stability in aseptically processed orange juice in TetraBrik cartons and the effect of oxygen. *Food Chemistry* 45:327-31.

- Klimant Ingo, Wolfbeis Otto S. 1995. Oxygen-Sensitive Luminescent Materials Based on Silicone-Soluble Ruthenium Diimine Complexes. *Analytical Chemistry* 67(18): 3160-66.
- Kothapalli A, Hayes K, Sadler G, Morgan M. 2007. Comparison of kinetic profile of free and immobilized glucose oxidase, immobilized on low-density polyethylene using UV polymerization. *Journal of Food Science* 72(9):C478-C82.
- Lee SK, Okura I. 1997. Optical sensor for oxygen using a porphyrin-doped sol-gel glass. *Analyst* 122(1):81-4.
- Lewis ME. 2006. National Field Manual. Chapter 6.2 Dissolved oxygen. In: (USGS) USGS, editor: Office of Water Quality.
- Mack TE, Heldman DR, Singh RP. 1976. Kinetics of oxygen-uptake in liquid foods. *Journal of Food Science* 41(2):309-12.
- Manso MC, Oliveira FAR, Oliveira JC, Frias JM. 2001a. Modelling ascorbic acid thermal degradation and browning in orange juice under aerobic conditions. *International Journal of Food Science and Technology* 36(3):303-12.
- Manso MC, Oliveira FAR, Oliveira JC, Frías JM. 2001b. Modelling ascorbic acid thermal degradation and browning in orange juice under aerobic conditions *Int J Food Sci Technol* 36(3):303-12.
- McDonagh C, MacCraith BD, McEvoy AK. 1998. Tailoring of sol-gel films for optical sensing of oxygen in gas and aqueous phase. *Anal Chem* 70:45-50.
- Mistry B, Min DB. 1992. Reduction of dissolved-oxygen in model salad dressing by glucose oxidase-catalase dependent on pH and temperature. *Journal of Food Science* 57(1):196-9.
- Ogurtsov VI, Papkovsky DB. 1998. Selection of modulation frequency of excitation for luminescence lifetime-based oxygen sensors. *Sensors and Actuators B-Chemical* 51(1-3):377-81.
- Ough CS. 1975. Further investigations with glucose oxidase-catalase enzyme-systems for use with wine. *American Journal of Enology and Viticulture* 26(1):30-6.
- Ovsejevi K, Grazu V, Batista-Viera F. 1998. beta-galactosidase from *Kluyveromyces lactis* immobilized on to thiol-sulfinate/thiol-sulfonate supports for lactose hydrolysis in milk and dairy by-products. *Biotechnology Techniques* 12(2):143-8.
- Parpinello GP, Chinnici F, Versari A, Riponi C. 2002. Preliminary study on glucose oxidase-catalase enzyme system to control the browning of apple and pear purees. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology* 35(3):239-43.

- Peleg H, Naim M, Zehavi U, Rouseff RL, Nagy S. 1992. Pathways of 4-Vinylguaiacol Formation from Ferulic Acid in Model Solutions of Orange Juice. *Journal of Agricultural and Food Chemistry* 40(5):764-7.
- Pickering GJ, Heatherbell DA, Barnes MF. 1998. Optimising glucose conversion in the production of reduced alcohol wine using glucose oxidase. *Food Research International* 31(10):685-92.
- Rassis D, Saguy IS. 1995. Oxygen effect on nonenzymatic browning and vitamin-c in commercial citrus juices and concentrate. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie* 28(3):285-90.
- Reyes-De-Corcuera JI, Cavalieri RP, Powers JR, Tang JM, Kang DH. 2005. Enzyme-electropolymer-based amperometric biosensors: An innovative platform for time-temperature integrators. *Journal of Agricultural and Food Chemistry* 53(23):8866-73.
- Ringblom U. 2004. *The orange book*. 2nd ed. Lund, Sweden: Tetra pak
- Roig MG, Bello JF, Velasco FG, Decelis CD, Cachaza JM. 1987. Applications of immobilized enzymes. *Biochemical Education* 15(4):198-208.
- Roy I, Gupta MN. 2003. Lactose hydrolysis by Lactozym (TM) immobilized on cellulose beads in batch and fluidized bed modes. *Process Biochemistry* 39(3):325-32.
- Roy I, Sardar M, Gupta MN. 2000. Exploiting unusual affinity of usual polysaccharides for separation of enzymes on fluidized beds. *Enzyme and Microbial Technology* 27(1-2):53-65.
- Sagi I, Mannheim CH. 1990. The effect of enzymatic oxygen removal on quality of unpasteurized and pasteurized orange juice. *Journal of Food Processing and Preservation* 14(4):253-66.
- Sankaran K, Godbole SS, Dsouza SF. 1989. Preparation of spray-dried, sugar-free egg powder using glucose-oxidase and catalase coimmobilized on cotton cloth. *Enzyme and Microbial Technology* 11(9):617-9.
- Shin DH, Cheigh HS, Lee DS. 2002. The use of Na₂CO₃-based CO₂ absorbent systems to alleviate pressure buildup and volume expansion of kimchi packages. *Journal of Food Engineering* 53(3):229-35.
- Shin MC, Kim HS. 1995. Effects of enzyme concentration and film thickness on the analytical performance of a polypyrrole glucose-oxidase biosensor. *Analytical Letters* 28(6):1017-31.
- Singh RP, Heldman DR, Kirk JR. 1976. Kinetics of quality degradation - ascorbic-acid oxidation in infant formula during storage. *Journal of Food Science* 41(2):304-8.

- Tang Y, Tehan EC, Tao ZY, Bright FV. 2003. Sol-gel-derived sensor materials that yield linear calibration plots, high sensitivity, and long-term stability. *Analytical Chemistry* 75(10):2407-13.
- Trammell DJ, Dalsis DE, Malone CT. 1986. Effect of oxygen on taste, ascorbic-acid loss and browning for htst-pasteurized, single-strength orange juice. *Journal of Food Science* 51(4):1021-3.
- Watkins A. Neal, Brett R. Wenner, Jeffrey D. Jordan, Wenying Xu, James N. Demas, Frank V. Bright. 1998. Portable, Low-Cost, Solid-State Luminescence-Based O₂ Sensor. *Applied Spectroscopy* 52 (5): 750-754.
- Yeh TS, Chu CS, Lo YL. 2006. Highly sensitive optical fiber oxygen sensor using Pt(II) complex embedded in sol-gel matrices. *Sensors and Actuators B-Chemical* 119(2):701-7.
- Zhou QZK, Chen XD. 2001. Effects of temperature and pH on the catalytic activity of the immobilized beta-galactosidase from *Kluyveromyces lactis*. *Biochemical Engineering Journal* 9(1):33-40.

BIOGRAPHICAL SKETCH

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