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MODELING OF RESPIRATION RATE OF OZONE TREATED MILKY MUSHROOMS

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Abstract: A respiration rate model, based on enzyme kinetics and the Arrhenius equation was proposed for predicting the respiration rates of Milky mushroom as a function of O2 and CO2 concentrations, storage temperature and ozone treatment. Temperature and ozone treatments were found to influence the model parameters. The enzyme kinetic model parameters, calculated from the respiration rate at different O2 and CO2 concentration were used to fit the Arrhenius equation against different storage temperature. The activation energy and respiration pre-exponential factor were used to predict the model parameters of enzyme kinetics at any storage temperature of 5–25°C. The developed models were tested for its validity at 10°C and it was found to be in good agreement (the mean relative deviation moduli between the predicted and experimental respiration rates were found to be 2.9% and 9.48% for O2 consumption and CO2 evolution, respectively) with the experimentally estimated respiration rates.
Keywords: Milky mushroom, Respiration rate, Enzyme kinetics, Modeling.

INTRODUCTION

Calocybe indica commonly known as milky mushroom is a well recognized tropical edible mushroom and promising for cultivation in India (Purkayastha and Chandra, 1976). This is the first indigenous mushroom to be commercialized in India. It contains highest protein (17.2%) and has 12 essential amino acids. Cropping requires an optimum temperature of 32-35°C, humidity of 85-90%, diffused light and ventilation. Biological efficiency potential for this mushroom is 50 – 100%. Shelf life of milky mushroom is 1-2 days at 25-30°C and 7-10 days at 5°C if microbial spoilage is taken care of. Browning, veil-opening, weight-loss and microbial spoilage are the most common postharvest changes in the mushrooms which often result in the reduction in shelf life of mushrooms. Accurate measurement of respiration rates and modeling is pivotal to the design of MA for agricultural commodity (Mangaraj and Goswami, 2011).

Talasila *et al.* (1992) developed a non-linear empirical model for predicting the respiration rate of strawberry as a function of temperature, O2 and CO2 concentration. Most of the model have not incorporated one or other dependent factors such as O2, CO2,

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temperature and time, and hence, are not flexible enough to predict the respiration rate at various storage conditions. The recent approach for modeling respiration rates by using Michaelis-Menten type equation is based on enzyme kinetics. This provides a simple description of respiration, based on the assumption that diffusion and solubility of O2 and CO2 in plant tissue regulates reactions catalyzed by enzymes (Lee *et al.,* 1991).

Ozone had been tested for the preservation of food and food ingredients such as milk, meat products, gelatin, casein, and albumin. Ozone applications in food industry are mostly related to decomposition of product surface and water treatment. Ozone has been used with success to inactivate contaminant microflora on meat, poultry, eggs, fish, fruits and vegetables and dry foods. Excessive use of ozone, however, may cause oxidation of some ingredients on food surface. This usually results in discoloration and deterioration of food flavor (Kim *et al.,* 1999). Treating fruits and vegetables with ozone has been used to increase shelf life (Norton et al., 1968; Rice *et al.,* 1982). Treatment of apples with ozone resulted in lower weight loss and spoilage (Bazarova, 1982).

Keeping this in view the present study was undertaken to study the effect of temperature and ozone pretreatment on respiration of milky mushroom.

MATERIALS AND METHODS

Produce and sample preparation

Fresh milky mushroom was harvested from local farm and packed in polyethylene bags for transportation. On arrival, mushroom were cleaned thoroughly to remove any foreign materials and wiped with clean dry cloth and stored in 5°C. Thereafter, the mushroom was used for the experiments.

Treatment for mushroom

Mushroom was subjected to gaseous ozone treatment in different concentrations (10, 20 and 30 ppm for 5 minutes). Ozone was produced using ozone generator (Oz-Air 5, Noida, India).

Gas exchange measurement

Closed system method was used for generating the respiration data for mushrooms at different temperatures (Amir *et al.* 2010). Air tight glass bottles of capacity 3L was used for the study. Mushrooms were kept in bottles and were closed with a lid and made it hermetically sealed using cilliputty. A septum was glued to the bottle for taking the gas sample. The setup was kept in humidity control chamber (Technico, Chennai, India), which

was maintained at desired temperatures with a variation of ± 0.5°C and relative humidity of 90±2% (Fig. 1). Experiments were conducted at 5, 15 and 25 ± 0.5°C temperatures.

Gas analysis

The head space oxygen and carbon dioxide concentrations within the glass bottles were monitored periodically using a portable O_2/CO_2 gas analyzer (PBI Dansensor), after calibrated with standard gases. Using the O_2 and CO_2 concentrations obtained at regular intervals, the respiration rates of sample were found out at different temperatures.

Fig. 1 Respiration study of mushrooms placed inside the humidity chamber

Free volume of the bottle

Free volume was determined by water displacement method (Deepak and Shashi, 2007). ***Modeling and data analysis***

The experimental respiration rates in terms of oxygen and carbon dioxide were calculated by using Eqns (1) and (2) as given by Kays (1991) and Mahajan and Goswami (2001).

$$R_{O2} = \left[\frac{(G_{O2})_t - (G_{O2})_{t+1}}{\Delta t}\right]\frac{V}{W} \quad (1)$$

$$R_{CO2} = \left[\frac{(G_{CO2})_{t+1} - (G_{CO2})_t}{\Delta t}\right]\frac{V}{W} \quad (2)$$

Where: R_{O2} and R_{CO2} are the respiration rate for oxygen consumption and carbon dioxide evolution, respectively in ml. $kg^{-1}h^{-1}$, G_{O2} and G_{CO2} are the gas concentrations for oxygen and carbon dioxide respectively in decimal, t is the storage time in h, Δt is the time difference between two gas measurements, V is the free volume of respiration chamber in ml and W is the weight of mushrooms in g. Two different approaches were used to model the respiration rate based on the experimental data as outlined below.

***Model I*:** By using the experimental respiration data, a non- linear regression analysis using Microsoft excel solver was used to fit the curves of oxygen and carbon dioxide stored in

different temperatures with treated and untreated samples. The equations used for curve fitting are shown in equation 3 and 4 to determine the values of the coefficients 'a' and 'b'. Mahajan and Goswami (2001) used the same equations for fitting the respiration rates.

$$G_{O2} = 0.21 - [\frac{t}{(at+b)}] \quad (3)$$

$$G_{CO2} = [\frac{t}{(at+b)}] \quad (4)$$

Where: a and b are the regression coefficients, t is the storage period in h, G_{O2} is the oxygen concentration in decimal and G_{CO2} is the carbon dioxide concentration in decimal. The first derivative with respect to time of the best fitted equation will give the respiration rate. Hence Eqns (3) and (4) were subjected to the first derivative with respective to time was outlined in Eqns (5) and (6) respectively.

$$\frac{\mathrm{d}(G_{O2})}{\mathrm{d}t} = at(at+b)^{-2} - (at+b)^{-1} \quad (5)$$

$$\frac{\mathrm{d}(G_{CO2})}{\mathrm{d}t} = at(at+b)^{-2} + (at+b)^{-1} \quad (6)$$

The respiration rate of the sample at any given time was then calculated by substituting the values of $d(G_{O2})/dt$ and $d(G_{CO2})/dt$ obtained from equations 5 and 6 in equation 7 and 8 respectively.

$$R_{O2} = -\frac{\mathrm{d}[G_{O2}]}{\mathrm{d}t}\frac{V}{W} \quad (7)$$

$$R_{CO2} = \frac{\mathrm{d}[G_{CO2}]}{\mathrm{d}t}\frac{V}{W} \quad (8)$$

Model II: Michaelis- Menten type equation (Eqns (9) and (10)) has been fitted to find the respiration rate which acts as the basic principle of the enzyme kinetics was the second model fitted to the experimental respiration data (Mahajan and Goswami 2001). In this carbon dioxide is considered as the inhibitor for the respiration and is not bind to the enzymes. But it is reversibly bind to the enzyme substrate complex. When the carbon dioxide concentration level reaches above 16- 17% the respiration changes from aerobic to anaerobic and that time this model is not valid (Mahajan and Goswami 2001). Michaelis- Menten equation for finding out respiration rates are:

$$R_{O2} = \frac{v_{m(O2)}G_{(O2)}}{k_{m(O2)}+\{1+([G_{CO2}]/k_{i(O2)})\}G_{O2}} \quad (9)$$

$$R_{CO2} = \frac{v_{m(CO2)}G_{(O2)}}{k_{m(CO2)}+\{1+([G_{CO2}]/k_{i(CO2)})\}G_{O2}} \quad (10)$$

Where: $k_{m(O2)}$ and $k_{m(CO2)}$ are the Michaelis Menten constants for O_2 consumption and CO_2 evolution, respectively, in % O_2; $k_{i(O2)}$ and $k_{i(CO2)}$ are the inhibition constants for O_2 consumption and CO_2 evolution, respectively, in % CO_2 ; $v_{m(O2)}$ and $v_{m(CO2)}$ are the maximum respiration rates for O_2 consumption and CO_2 evolution, respectively, in ml kg^{-1} h^{-1}; R_{O2} is the respiration rate in ml [O_2] kg^{-1} h^{-1} and R_{CO2} is the respiration rate in ml [CO_2] kg^{-1} h^{-1}; and G_{O2} and G_{CO2} are the concentrations of oxygen and carbon dioxide, respectively.

The temperature dependence of the model parameters of the above Michaelis- Menten equations were quantified using an Arrhenius type equation (11).

$$R_m = R_p \exp\left[\frac{-E_a}{R \times T}\right] \quad (11)$$

Where: R_m is the model parameter of Michaelis- Menten equation; R_p is the respiration pre-exponential factor; E_a is the activation energy in kJ $g^{-1}mol^{-1}$; T is the storage temperature in K and R is the universal gas constant in kJ $g^{-1}mol^{-1}K^{-1}$(8.314 kJ $g^{-1}mol^{-1}K^{-1}$). The above equation can be expressed in a linearised form as follows:

$$lnR_m = -\frac{-E_a}{R}\left[\frac{1}{T}\right] + \ln R_p \quad (12)$$

Verification of the model

Respiration rates of mushroom predicted by models were verified with experimental respiration rates at 5°C storage temperature. The goodness of fit between experimental and predicted respiration rates was obtained by using the mean relative deviation modulus (E) as given in equation (13). According to McLaughlin and O'Beirne, 1999 moduli below 10% are representing a reasonable good fit, between 10 and 20% are fairly good fit and between 20 and 30% are of not satisfactory fit for all practical purposes.

$$E = \left[\frac{100}{N}\sum_{i=1}^{n}\frac{(R_{exp} - R_{pre})}{R_{exp}}\right] \quad (13)$$

Where: E is the mean relative deviation modulus in %; N is the number of respiration data points; R_{exp} is the experimental respiration rate in ml $kg^{-1}h^{-1}$ and R_{pre} is the predicted respiration rate in ml $kg^{-1}h^{-1}$.

RESULTS AND DISCUSSIONS

The respiration rate of ozone treated and untreated samples were measured experimentally and models were developed to predict the same at any temperature in the range of 5 to 25°C.

Free volume and weight

Free volume of the mushroom holding glass bottles and the weight of the mushroom kept in the respective glass bottles are shown in table 1.

Experimental respiration data

Figure 2 shows the actual gas composition in terms of oxygen and carbon di oxide inside the glass bottles with different ozone treatments kept in different temperatures. From the obtained graphs it is observed that as the temperature increased, the respiration also increased in a faster rate. This is due to the change in the respiration rate which is influenced by the storage temperature. The time taken for reaching the least oxygen concentration and the high carbon dioxide concentration is more in 30ppm ozone treated samples. This shows that the ozone treatment is having an effect in controlling the respiration rate of mushrooms. The maximum time taken for reaching the least oxygen content of 0.5% is 2520 minutes, in which the sample was kept in 5°C and treated with 30ppm ozone. Minimum time taken (660 minutes) is by the mushrooms kept in 25°C without any treatment. In all temperatures untreated samples were kept to observe the variation in the results.

Estimation of parameters for different models

Model I

The regression coefficients a and b of equations 3 and 4 and their corresponding r^2 (coefficients of determination) values at different storage temperatures were determined for ozone treated and untreated mushrooms and are shown in table 2. The experimental data fitted very well with the regression equation having r^2 values > 0.854 indicates that the regression functions fit the data very well. From the values of the regression coefficients a and b as shown in table 2, it can be inferred that both parameters were influenced by the storage temperature and ozone treatment (Mangaraj and Goswami, 2011). However, coefficient b was more influenced than coefficient a.

Model II

The model parameters in equation 9 and 10 were estimated by fitting the experimental data of each temperature and ozone treatment using MS Excel Solver. The model parameters were calculated and are given in table 3 along with their corresponding coefficients of

determination (r^2) values. With the help of these model parameters, respiration rates can be predicted using Eqns. 9 and 10 with any combination of O_2 and CO_2 concentration respectively.

Table 1: Free volume and the weight of the mushroom kept for respiration rate study

Storage temperature, °C	Ozone Conc., ppm	Weight (W), g	Volume (V), ml
	Untreated	675	2320
	10	605	2100
	20	630	2184
5	30	645	2312
	Untreated	635	2220
	10	610	2132
	20	630	2190
15	30	615	2170
	Untreated	610	2150
	10	595	2090
	20	635	2215
25	30	645	2222

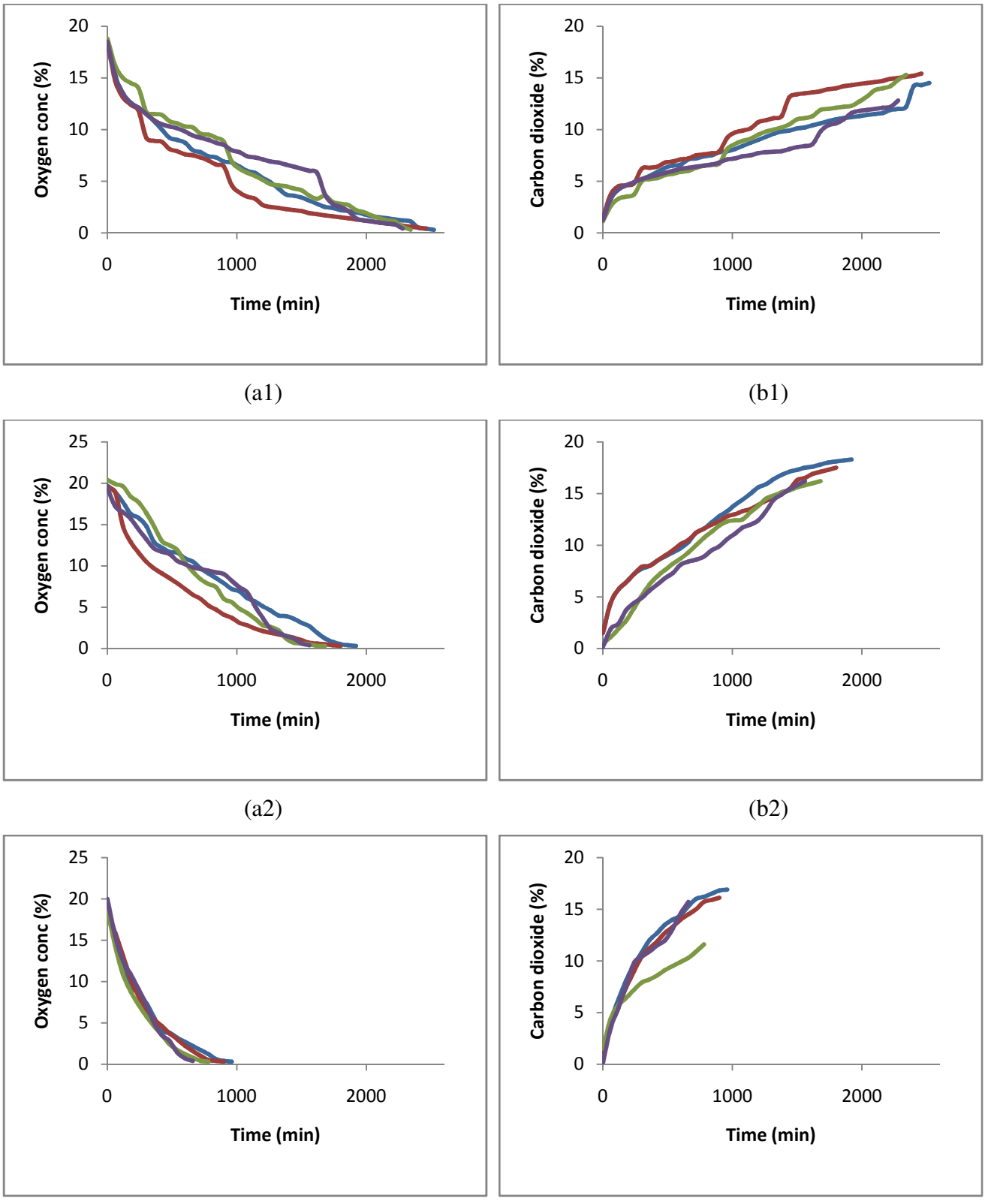

Figure 2: Changes in the concentration for (a1, a2, a3) oxygen and (b1, b2, b3) carbon di oxide kept in different storage temperatures (1:5°C, 2:15°C, 3:25°C); WO: without treatment, 10: 10 ppm treated, 20: 20 ppm treated, 30: 30 ppm treated

Table 2: Regression coefficients a and b for oxygen consumption and cabon dioxide evolution respectively, for treated and untreated samples stored in different temperatures

Storage Temperature	Ozone treatment	Respiration expression in terms of	Regression Coefficients		r^2
			a	b	
5	Without treatment	O_2 Consumption	4.31	2554.66	.964
		CO_2 evolution	6.27	6222.96	.913
	10 ppm	O_2 Consumption	3.81	2981.39	.934
		CO_2 evolution	3.67	7970.08	.966
	20 ppm	O_2 Consumption	4.28	1597.08	.900
		CO_2 evolution	4.22	5606.20	.968
	30 ppm	O_2 Consumption	4.24	2098.12	.854
		CO_2 evolution	5.98	5303.46	.923
15	Without treatment	O_2 Consumption	2.47	4082.78	.965
		CO_2 evolution	2.41	6269.61	.877
	10 ppm	O_2 Consumption	1.78	4669.43	.987
		CO_2 evolution	3.14	4918.75	.909
	20 ppm	O_2 Consumption	3.79	1873.96	.915
		CO_2 evolution	4.44	2934.81	.882
	30 ppm	O_2 Consumption	2.67	4237.02	.892
		CO_2 evolution	3.73	3308.37	.851
25	Without treatment	O_2 Consumption	3.04	1165.70	.952
		CO_2 evolution	4.33	1632.20	.953
	10 ppm	O_2 Consumption	3.81	771.64	.953
		CO_2 evolution	7.73	1344.91	.952
	20 ppm	O_2 Consumption	3.64	1008.9	.900
		CO_2 evolution	4.45	1594.57	.943
	30 ppm	O_2 Consumption	3.88	920.13	.916
		CO_2 evolution	5.20	1203.59	.943

Table 3: Model parameters of uncompetitive inhibition enzyme kinetics for different storage temperatures and ozone treatments

Storage Temperature	Ozone treatment	Respiration expression in terms of	Maximum respiration rate (V_m), ml/kg h	Michaelis-Menten constant (K_m), $\%O_2$	Inhibition constant (Ki), $\%CO_2$	R^2
5	Without treatment	O_2 Consumption	7.45	9.69	0.02	0.965
		CO_2 evolution	3.33	3.24	0.02	0.877
	10 ppm	O_2 Consumption	2.82	15.19	0.06	0.995
		CO_2 evolution	1.45	24.06	0.10	0.916
	20 ppm	O_2 Consumption	1.01	9.73	0.02	0.874
		CO_2 evolution	1.47	6.87	0.17	0.848
	30 ppm	O_2 Consumption	2.07	9.38	0.01	0.960
		CO_2 evolution	1.20	5.92	0.52	0.903
15	Without treatment	O_2 Consumption	3.60	10.67	0.04	0.878
		CO_2 evolution	0.99	2.48	0.25	0.891
	10 ppm	O_2 Consumption	0.66	15.25	0.74	0.927
		CO_2 evolution	0.11	3.69	0.48	0.903
	20 ppm	O_2 Consumption	6.1	9.78	0.44	0.897
		CO_2 evolution	8.66	1.89	0.43	0.973
	30 ppm	O_2 Consumption	8.04	1.99	0.13	0.888
		CO_2 evolution	2.40	1.34	0.52	0.965
25	Without treatment	O_2 Consumption	3.6	10.48	0.85	0.863
		CO_2 evolution	3.0	2.91	0.17	0.964
	10 ppm	O_2 Consumption	12.43	7.95	0.30	0.976
		CO_2 evolution	7.65	7.95	0.04	0.962
	20 ppm	O_2 Consumption	10.42	9.78	0.10	0.940
		CO_2 evolution	1.18	1.45	0.34	0.951
	30 ppm	O_2 Consumption	2.23	2.09	0.95	0.981
		CO_2 evolution	9.44	4.83	0.52	0.929

Table 4: Analysis of variance of effect of temperature and ozone treatments on model parameters of enzyme kinetics

Factor	DF	$V_{m(O2)}$		$V_{m(CO2)}$		$K_{m(O2)}$		$K_{m(CO2)}$		$K_{i(O2)}$		$K_{i(CO2)}$	
		MSS	F value	MSS	F value	MSS	F value	MSS	F value	MSS	F value	MSS	F value
Ozone treatment	12	82.24	1.16	87.46	1.32	48.33	.54	64.05	.65	71.00	1.02	70.98	1.02
Temperature	3	397.13	5.61	537.62	8.12	128.52	1.45	357.02	3.8	863.77	12.49	864.75	12.51

Table 5: Slope and Y axis intercept of Arrhenius relation for different model parameters of enzyme kinetics

Ozone Treatment		V_m		K_m		K_i	
		O_2	CO_2	O_2	CO_2	O_2	CO_2
Without treatment	Slope	-2978	-4998	-396	-1111	1542	1056
	Y axis intercept	11.86	18.13	0.952	4.91	-51.12	-34.31
10 ppm	Slope	-8684	-7690	-2734	-7758	1045	1027
	Y axis intercept	31.48	28.24	12	29.13	-34.87	-33.59
20 ppm	Slope	-9744	-8186	-210	-6398	1282	3872
	Y axis intercept	35.23	29.33	2.35	23.2	-42.2	-12.21
30 ppm	Slope	-5565	-8519	-6355	-6217	1891	653
	Y axis intercept	20.53	30.69	23.29	22.8	-63.46	-45

Table 6: Activation energy and pre-exponential factor of Arrhenius type equation for different model parameters of uncompetitive enzyme kinetics

Ozone Treatment	Parameters for Arrhenius equation	V_m		K_m		K_i	
		O_2	CO_2	O_2	CO_2	O_2	CO_2
Without treatment	E_a (kJ/g-mole)	24.76	41.55	3.29	9.24	-12.82	-8.78
	R_p	1.4×10^6	7.4×10^8	2.5×10^8	1.3×10^2	1.5×10^6	7.9×10^4
10 ppm	E_a (kJ/g-mole)	72.20	63.93	22.73	64.50	-8.69	-8.54
	R_p	4.6×10^{13}	1.8×10^{12}	1.6×10^5	4.4×10^{12}	1.3×10^5	3.8×10^4
20 ppm	E_a (kJ/g-mole)	81.01	68.06	1.75	53.19	-10.66	-32.19
	R_p	1.9×10^{15}	5.4×10^{12}	1.0×10^7	1.1×10^{10}	2.1×10^8	2×10^6
30 ppm	E_a (kJ/g-mole)	46.27	70.83	52.84	51.69	-15.72	-5.43
	R_p	8.2×10^8	2.1×10^{13}	1.3×10^{10}	7.9×10^9	3.6×10^7	3.4×10^9

The value of table 3 shows that, model II parameters were also dependent on the storage temperature and ozone treatment. Coefficient of determinants of this model is indicating that the relationship between respiration rate and oxygen and carbon dioxide concentrations is fitted well.

The analysis of variance of temperature and ozone treatment with model parameters of enzyme kinetic model is listed in table 4. It can be inferred that the temperature has a significant effect (5% level of significance) on the model parameters v_m, k_m and k_i for O_2 and CO_2 concentrations of enzyme kinetic model (model 2).

Fitting Arrhenius equation

The model parameters such as v_m, k_m and k_i for O_2 and CO_2 concentrations, were found to vary with the temperature hence Arrhenius equation was used to co- relate the model parameters at different storage temperatures and ozone treatments. As per the equation 12 the model parameters were plotted at different storage temperatures for ozone treated and non-treated mushroom samples; by plotting the log values of the model parameters against the inverse of corresponding temperature in absolute temperature. The slope and Y- axis intercept of equation 12 for model parameters v_m, k_m and k_i are shown in table 5. The activation energy was calculated from the slope of the straight line and the pre-exponential factor was calculated from the Y-axis intercept. Table 6 shows the activation energy and pre-exponential factor for different model parameters of enzyme kinetics. Activation energy was found to be in negative side for k_i (O_2) and k_i (CO_2). The negative values of activation energies for ki could be attributed to the inhibitory effect of CO_2 concentration on respiration rate. By using these constants, the model parameters at any temperatures can be predicted by using equation 12 and then, the respiration rate at the given temperature can be estimated for respiration rates in terms of O_2 consumption and CO_2 evolution, respectively.

Verification of respiration rate models

The respiration study was done at temperatures of 5, 15 and 25°C for ozone treated and non-treated oyster mushrooms. So the respiration rates were generated at these temperatures. However, the developed models were verified to assess the capability of its predictability of the respiration rates at ant temperature between 5 and 25°C. The respiration rate models were verified at 10°C storage temperature for non treated mushrooms. For generating the experimental data for O_2 and CO_2, the free volume of the chamber and the weight of the mushrooms were as 2320 ml and 675g, respectively. Figure 2 shows the change in O_2 and CO_2 concentrations with storage time at 10°C. Closed respiration system was used

to obtain the respiration rates. The regression coefficients a and b at 10°C storage temperature was found out using the regression model (model 1). The respiration rates of mushroom in terms of O_2 consumption and CO_2 evolution were estimated by using equations 9 and 10.

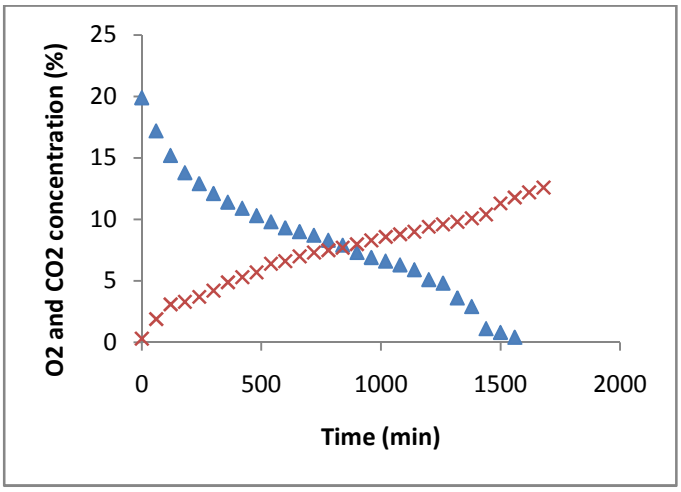

Figure 3: Plot of gas composition of mushroom at 10°C

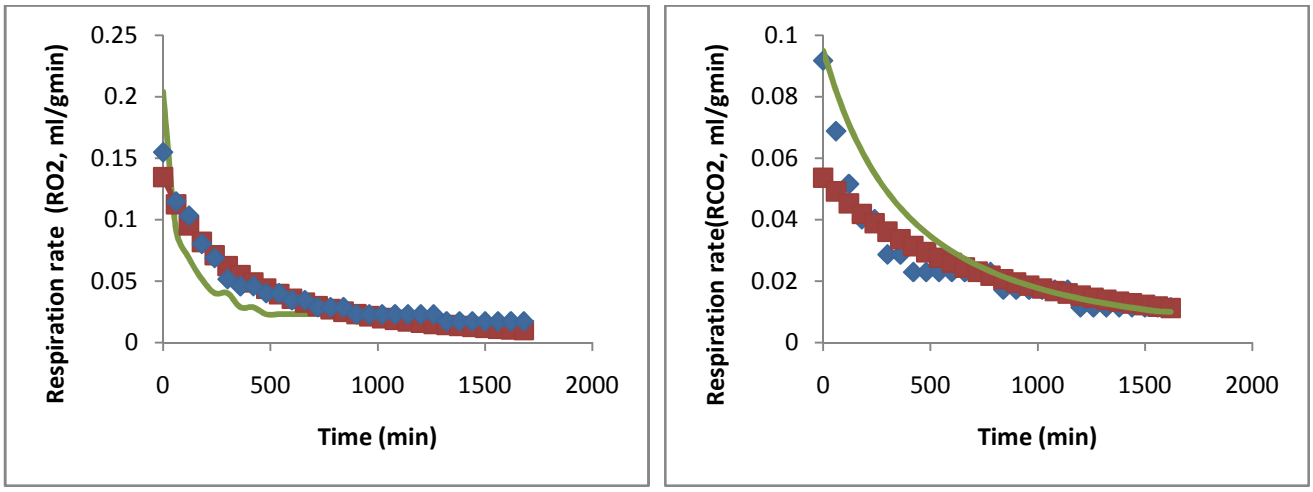

Figure 4: Predicted and experimental respiration rates of mushroom at 10°C storage temperature

The experimental respiration rates and respiration rates predicted by model 1 and model 2 for O_2 consumption and CO_2 evolution at 10°C are shown in figure 3. The mean relative deviation moduli (equation 13) between respiration rates of mushroom at 10°C predicted by regression analysis (model 1) and that obtained through experiments were 10.6% and 7.24% for O_2 consumption and CO_2 evolution, respectively. Similarly the mean relative deviation moduli between respiration rates of mushroom at 10°C predicted by enzyme kinetics (model 2) and that obtained through experiments were found to be 2.9% and 9.48% for O_2 consumption and CO_2 evolution, respectively. This suggested that the predicted respiration

rates for ozone treated and non- treated mushroom were close agreement with the experimental respiration rates.

CONCLUSION

Respiration rates for ozone treated and non- treated mushrooms at different temperatures from 5 to 25°C in step of 10°C were estimated using a closed system method. The respiration data generated by this method can be used to model the respiration rate. The respiration rates predicted by the regression model and enzyme kinetic model were found to be in close agreement with those obtained experimentally. In the enzyme kinetic model the dependence of respiration rates on O_2 and CO_2 was found to follow the uncompetitive inhibition. Predicted respiration rates by the models were found to be in good agreement with the experimental respiration rates. The activation energy and respiration pre-exponential factor could be used to predict the model parameter of enzyme kinetics at any storage temperature. There was a good agreement between experimental and predicted respiration rate at 10°C storage temperature.

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