

The Development of a Model to Describe the Influence of Temperature and Relative Humidity on Respiration Rate of Prickly Pear Cactus Stems in Reduced O₂ Conditions

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Abstract

Respiration rate (R_{O_2}) of prickly pear cactus stems (*Opuntia* spp.) was measured as a function of 4 temperature (T) and 6 relative humidity (RH) combinations for O₂ partial pressures between 15 and 0.8 kPa, which were considered to support aerobic respiration. The rate of respiration (R_{O_2}) was determined based on O₂ depletion of the atmosphere in sealed containers containing 1 kg of stems. The O₂ partial pressure declined linearly over time and the slopes of the fitted lines were used to calculate the rate of O₂ uptake. The rate of O₂ uptake increased with increasing temperature and decreased with increasing RH. The respiratory rate at 25°C was approximately 30 to 40 times higher than at 5°C. The respiratory rate at 65% RH was between 30 and 90% greater than at 90% RH, depending on the temperature. Data for $\ln(R_{O_2})$ for each RH level were regressed against the inverse of the T (K⁻¹) to determine Arrhenius constants and calculate the apparent E_a of respiration for the six RH conditions. The E_a was similar for each RH level, varying between a low of 113 to a high of 131 kJ·mol⁻¹. An equation having an R² of 0.95 was developed describing respiration as a function of RH and T (°C) using only four constants.

INTRODUCTION

Microbial contamination and physiological changes limit post-harvest shelf life of prickly pear cactus stems in refrigeration to approximately 6 days for stems with thorns and 2 days for stems without thorns (Guevara et al., 2001, 2003b). Modified atmosphere packaging (MAP) can inhibit post-harvest spoilage and has been shown to extend the cactus stem shelf-life for up to 30 days at 5°C (Guevara et al., 2001, 2003b). Optimum ranges for gas concentrations can be determined from physicochemical analysis and/or sensory tests, once appropriate limiting quality features are defined. Once optimum storage conditions are defined, needed package and film characteristics or features may be derived. Designing MAP systems, especially those that compensate for temperature changes during the postharvest holding period, requires knowledge of product (respiration rate, optimum gases concentration during storage), package (surface area and film thickness and permeability), and storage conditions (temperature and relative humidity).

It is well recognized that the respiratory demand of fresh, harvested products is a function of temperature, O₂ availability, and in some cases, CO₂ concentration. Typically, low temperature, low O₂ levels, and high CO₂ levels cause a decrease in enzymatic activity, thus, reducing the uptake of O₂ and the production of CO₂. However, although water is liberated in the process of respiration and water vapor loss is associated with the dissipation of respiratory and sensible heat, especially in leafy tissues, the impact of water vapor in the external atmosphere on respiratory activity has been little studied. Different equations have been developed to correlate respiration rate with different storage parameters such as O₂ and CO₂ concentrations (Salvador et al., 2002), but to our knowledge, the impact of the humidity of the storage environment on respiration has not been described mathematically.

A convenient way to measure respiration is to seal the plant product in containers and monitor gas concentrations in that container. Yang and Chinnan (1988), Cameron et al. (1989) and Talasila et al. (1992) used closed systems to measure and model respiratory activity under various temperature and atmospheric conditions. The closed system provides the additional benefit of permitting humidity modification using salt solutions within the sealed respiratory chamber. To date, no study has been performed to describe the aerobic respiratory activity of prickly pear cactus stems as a function of humidity and temperature.

The objectives of this work are to measure the aerobic respiration rate of prickly pear cactus stems as a function of temperature and RH within a range of non-limiting O₂ levels and to develop a mathematical function describing that relationship for prickly pear cactus stems.

MATERIALS AND METHODS

Prickly pear cactus (*Opuntia* spp.) stems (approx. 40 days from budding) were obtained from a local market in Querétaro, Mexico and were immediately brought to the laboratory and selected by size, uniformity and freedom from defects.

Oxygen uptake was monitored using a closed system (Guevara et al., 2003a; Hagggar et al., 1992; Yahia et al., 2005) at 65–90% RH (at 5% intervals) and at 5, 14, 20 and 25°C. Sealed 4-L glass jars equipped with sampling ports (one per condition), each one with approximately 1 kg of cactus stems and 25 ml of different saturated saline solutions. The saline solutions were used to produce different RH; chambers were equilibrated for 12 h before each assay in temperature-controlled rooms. Changes in O₂ and CO₂ levels were monitored periodically using a NITEC portable O₂/CO₂ gas analyzer, model GA-20 in a configuration that recirculated headspace gases. The data for the decline in the headspace oxygen as a function of holding period was fitted to a linear function to determine the rate of O₂ uptake for each of the 24 T/RH combinations. Only data considered to be a result of aerobic respiration were used; some initial data points, apparently associated with the adjustment of the measuring system were not used in the analysis. Data for CO₂ accumulation in the chamber headspace were not used for calculating respiratory activity because of concerns regarding the solubility of CO₂ in the cladodes, and to a lesser extent, in the salt solutions.

Once O₂ uptake rates were determined, the data were transformed using the natural logarithm to normalize variance and to more evenly weight the data across the range of temperatures and thereby improve fit at the lower temperature. The natural logarithm of respiration was regressed against RH using linear curves for each of the four holding temperature. Intercept and slope constants for all four curves were regressed against temperature and fitted lines were used to create a model for all the data as a function of temperature (°C) and RH (%). Respiratory data were un-transformed and reported as ml·kg⁻¹·hr⁻¹. The natural logarithm of the respiratory data was also regressed against the inverse of temperature (K⁻¹) for each of the six RH to determine Arrhenius constants including the apparent energy of activation, E_a. Statistical analysis was done using statistical analysis software for PC (Excel, Microsoft Corp.).

RESULTS AND DISCUSSION

The rate of O₂ uptake declined linearly with time for each of the 24 T/RH combinations of 5, 14, 20 and 25°C and 65, 70, 75, 80, 85 and 90% RH (Fig. 1). The r² for fitted linear lines ranged from a low of 0.98 to a high of 0.99 (Table 2). For all temperatures tested, the changes in O₂ (and CO₂) profiles for 85 and 90% RH were not different from one another, but differed markedly for depletion profiles for 65% and 70% RH. Linear changes in O₂ partial pressure agree with the expected pattern of a linear decline for many products held in closed containers under aerobic conditions including tomato (*Lycopersicon esculentum* Mill.) by Cameron et al. (1989), fresh green onions (*Allium fistulosum* L.) by Hong and Kim (2001), and for different horticultural products by Jacxsens et al. (2000). Non-linearity in the decline of the atmospheric O₂ level can be indicative of developmentally-dependent changes in the aerobic respiratory rate or the suppression of respiratory activity due to limiting O₂ partial pressures. Several models have described O₂ uptake as a function of O₂ including partial pressures in the range capable of inhibiting the terminal oxidases in the electron transport pathway (Cameron et al., 1994; Hayakawa et al., 1975; Mannapperuma et al., 1989).

Even as the O₂ partial pressures in this study did not apparently impact O₂ depletion rate, the CO₂ levels, which accumulated to partial pressures between 15 and 18 kPa by the conclusion of the study, also seemed to have had no impact on the O₂ content of the containers. Data for CO₂ inhibition of respiration in prickly pear cactus stems are not in the literature, and in fact, there is little to support a universal inhibition of CO₂ on O₂ uptake. Beaudry (1993) found that CO₂ did not reduce O₂ uptake of blueberry (*Vaccinium corymbosum* L.) fruit even at partial pressures from 20 to 60 kPa CO₂. Peppelenbos et al. (1993) noted little effect of CO₂ on O₂ uptake by mushrooms (*Agaricus bisporus* L.), and no effect of <20 kPa CO₂ on O₂ uptake was also noted by Joles et al. (1993) for several fruits. Kubo et al. (1990) measured the impact of CO₂ on O₂ uptake for 18 fruit and vegetables, several of which had no response or experienced an increase in O₂ consumption. They suggested that high CO₂ levels decreased or increased the respiration rate via its impact on ethylene biology. A more thorough exploration of the response of prickly pear respiration in response to CO₂ will be needed to conclusively demonstrate the impact.

The slope of the linear equations (Table 2) applied to the O₂ depletion curves yielded respiratory rates for the 24 T/RH combinations. R_{O₂} increased with temperature, but, at each temperature, declined with an increasing RH (Table 2). O₂ consumption increased 7- to 10-fold between 5 to 14°C, and approximately 5-fold between 14 to 25°C, which may be a result of a shift in physiological factors limiting metabolism in cactus stems. Similar results were obtained by Jacxsens et al. (2000) for broccoli florets. The extent of change in respiration with RH differed somewhat for the four temperature regimes; the increase in respiration was approximately 30, 60, 90 and 90% for 5, 14, 20 and 25°C, respectively. Curves fitted to respiratory data revealed that the increase in respiration with declining RH was roughly linear for each temperature (Table 2). The slopes for these fitted lines declined, and the intercepts increased, with increasing temperature. The relatively regular change in intercept and slope with temperature enabled fitting these data to linear curves; one describing the change in slope with temperature, and the other describing the change in the intercept with temperature (data not shown). When these equations were combined with the equation for changing respiration with humidity, they yielded a close fit between experimental and predicted values (R² = 0.97), however, the predictive curves were not accurate at the lowest temperature (data not shown). For this reason, the data were transformed using the natural log of the respiratory data and the inverse of temperature (°K) as indicated in Table 3 to provide greater statistical weighting to the lower temperature data. Equations regressing ln(R_{O₂}) against RH for each temperature resulted in four lines with r² values ranging between 0.78 and 0.90 (Table 4). The constants for these lines were found to vary with temperature linearly (Table 4, right portion). Weighting the respiratory rates by using the

natural logarithm eliminated the problem of inaccuracy at the lowest temperature, while lowering the fit only marginally ($R^2 = 0.95$, Fig. 3) and resulted in the following equation:

$$\ln(R) = \left(-0.1985 + 50.96 \times \left(\frac{1}{273.15 + T} \right) \right) \times RH + 67.64 - 18430 \times \left(\frac{1}{273.15 + T} \right)$$

where T is temperature in °C. Using this equation, the rate of O₂ uptake can be reasonably well predicted across a range of temperatures and relative humidity. To our knowledge, no previous study has integrated the effect of RH and T in a predictive model.

The Arrhenius equation is typically applied to describe the temperature dependence of different biological reactions, and, like the Q₁₀, has been used to model the temperature responsiveness of respiration of fresh horticultural crops (Cameron et al., 1995). The values of $\ln(R)$ regressed against T^{-1} (T in °K) yielded essentially linear relationships with similar slopes (Fig. 2). There was no indication that the slope of the Arrhenius relationship varied with RH (Table 3). The apparent activation energy (E_a) of the six relationships varied between 113 to 131 kJ·mole⁻¹ and is a function of the slope of the Arrhenius relationship (slope = $-E_a/R$, where $R = 0.0083144$ kJ·mole⁻¹·K⁻¹). The average E_a was approximately 120 kJ·mole⁻¹, which is relatively high compared to published values of 95, 55, 60 and 60 kJ·mole⁻¹ for broccoli, lettuce, blueberry, and strawberry, respectively (Cameron et al., 1995).

The relation of $\ln(R_{O_2})$ and T^{-1} (T in °K) for experimental values may not be fully linear; there may be a change in the slope at 14°C, but additional temperature points would be needed to verify changing slope (Fig. 2). Hagggar et al. (1992) and Song et al. (1992) observed a similar phenomenon. Cameron, et al. (1994) also reported a higher apparent E_a at lower temperature; they also noted that the apparent E_a increased 1.5- to 2-fold as oxygen increased from levels that limited respiration to non-limiting levels.

CONCLUSIONS

The natural log of the oxygen consumption rate was described by a linear model dependent on RH in which the slope and constant were linear relationships with temperature as follows:

$$\ln(R) = \left(-0.1985 + 50.96 \times \left(\frac{1}{273.15 + T} \right) \right) \times RH + 67.64 - 18430 \times \left(\frac{1}{273.15 + T} \right)$$

and by Arrhenius equations for each humidity level, which varied linearly with the inverse of temperature (K). Parameters from the respiration model and kinetic data may be useful in designing effective packaging models for perishable horticultural products such as prickly pear cactus stems.

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Tables

Table 1. Nomenclature and abbreviations used.

Abbreviation	Description	Units
R	Respiration rate	ml·kg ⁻¹ ·h ⁻¹
RH	Relative humidity	%
T	Temperature	°C or Kelvin
W	Fruit weight	kg
V	Free volume	L
t	Time	h
Ea	Activation energy	kJ·mol ⁻¹

Table 2. Values for slope (m), intercept (b) and r^2 for linear best fit curves fitted to data in Figure 1 for container oxygen as a function of holding time at 24 different temperature/relative humidity combinations; the slope (O_2 uptake) units are in $ml \cdot kg^{-1} \cdot h^{-1}$. Also, linear best fit values (slope, intercept, and r^2) for O_2 uptake data in columns regressed against relative humidity for each of the four temperatures.

RH (%)	Temperature ($^{\circ}C$)											
	5			14			20			25		
	m	b	r^2	m	b	r^2	m	b	r^2	m	b	r^2
65	1.17	12.4	0.99	11.4	12.5	0.99	27.8	14.6	0.98	51.1	13.3	0.99
70	1.17	13.7	0.99	10.1	12.3	0.99	25.8	14.5	0.98	39.0	12.8	0.99
75	1.14	14.4	0.99	8.29	12.0	0.98	16.7	13.2	0.99	33.9	12.7	0.99
80	1.09	15.1	0.99	7.21	12.0	0.99	15.2	13.6	0.99	29.0	12.1	0.98
85	0.84	14.1	0.99	6.66	11.9	0.99	15.3	13.6	0.99	28.2	12.6	0.99
95	0.89	15.0	0.99	6.94	12.1	0.99	14.8	13.5	0.99	26.9	12.7	0.99
Constants for best fit lines fitted to above slope data												
Slope	-0.01409			-0.1939			-0.5598			-0.9040		
intercept	2.142			23.47			62.66			104.7		
R^2	0.79			0.88			0.79			0.85		

Table 3. To the left, natural logarithm of oxygen uptake of prickly pear stems in Table 2 for 24 different relative humidity/temperature combinations. Also, to the right, linear best fit constants for r^2 , intercept, and slope for $\ln(R)$ data regressed against the inverse temperatures (Kelvin) for each of the six humidity levels, by row. Units for the apparent energy of activation (Ea) are $kJ \cdot mol^{-1}$.

RH (%)	1/Temperature (K^{-1})				Arrhenius constants			
	0.00360 (10 $^{\circ}C$)	0.00348 (15 $^{\circ}C$)	0.00341 (20 $^{\circ}C$)	0.00335 (25 $^{\circ}C$)	Slope	Intercept	r^2	Ea (kJ/mol)
65	0.159	2.44	3.33	3.93	-15743	56.9	0.98	131
70	0.157	2.32	3.25	3.66	-14832	53.7	0.97	123
75	0.130	2.12	2.81	3.52	-13940	50.4	0.98	116
80	0.0845	1.98	2.72	3.37	-13573	49.0	0.99	113
85	-0.177	1.90	2.73	3.34	-14632	52.6	0.98	122
90	-0.118	1.94	2.69	3.29	-14163	50.9	0.98	118

Table 4. Linear best fit constants (slope, intercept, and r^2) for $\ln(R)$ data in Table 3 regressed against relative humidity for each temperature. Also, to the right, constants for lines fitted to the slope and intercept constants, by row.

	1/Temperature (K^{-1})				Change in constants with 1/T		
	0.00360 (10 $^{\circ}C$)	0.00348 (15 $^{\circ}C$)	0.00341 (20 $^{\circ}C$)	0.00335 (25 $^{\circ}C$)	Slope	Intercept	r^2
Slope	-0.01391	-0.0222	-0.0275	-0.0248	50.96	-18430	0.81
Intercept	1.117	3.837	5.053	5.440	0.953	67.64	0.96
r^2	0.78	0.89	0.80	0.90			

Figures

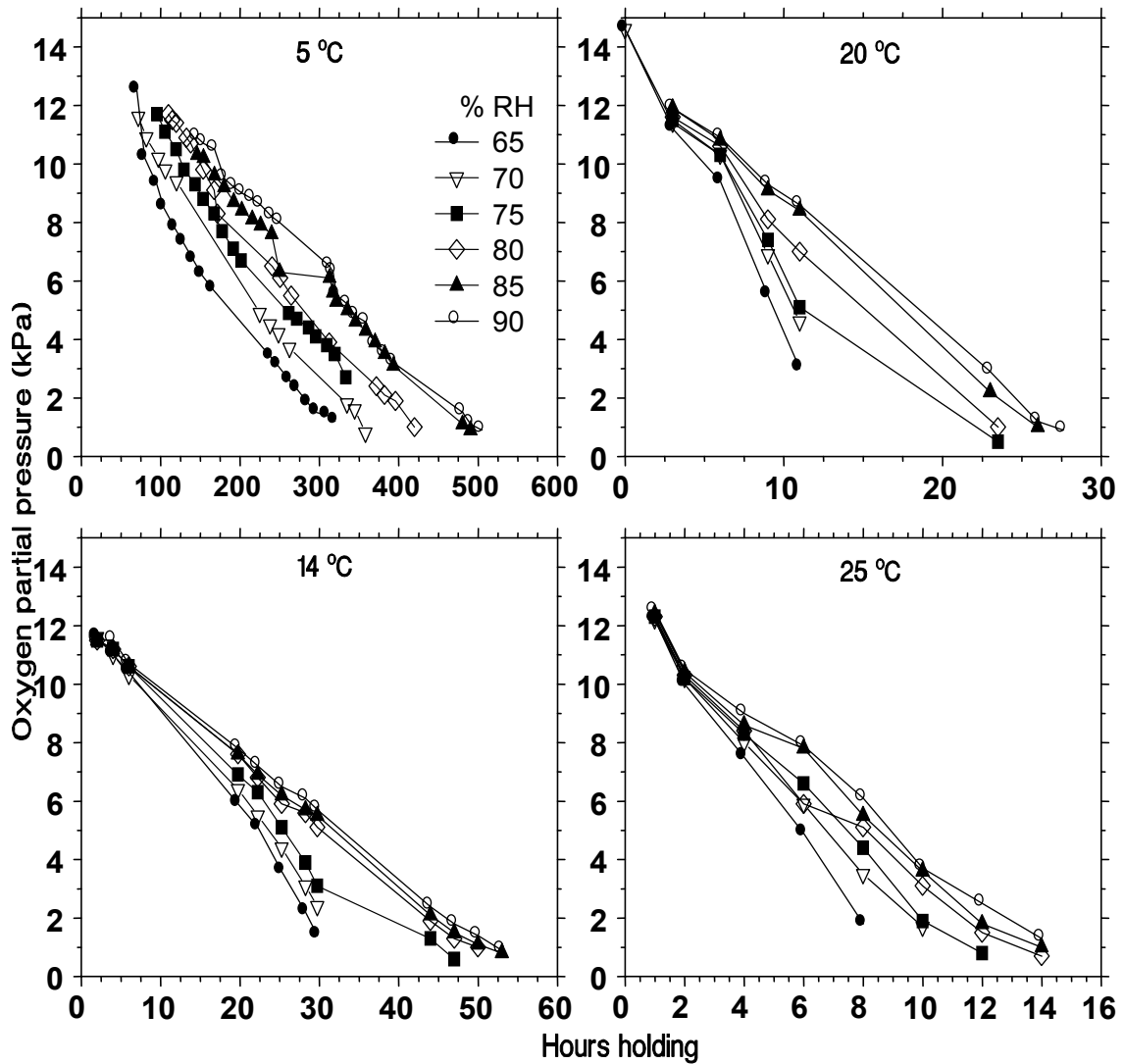


Fig. 1. Changes in headspace oxygen partial pressure (kPa) as a function of different temperatures and relative humidity. (●) 65% RH, (▽) 70% RH, (■) 75% RH, (◇) 80% RH, (▼) 85% RH and (○) 90% RH.

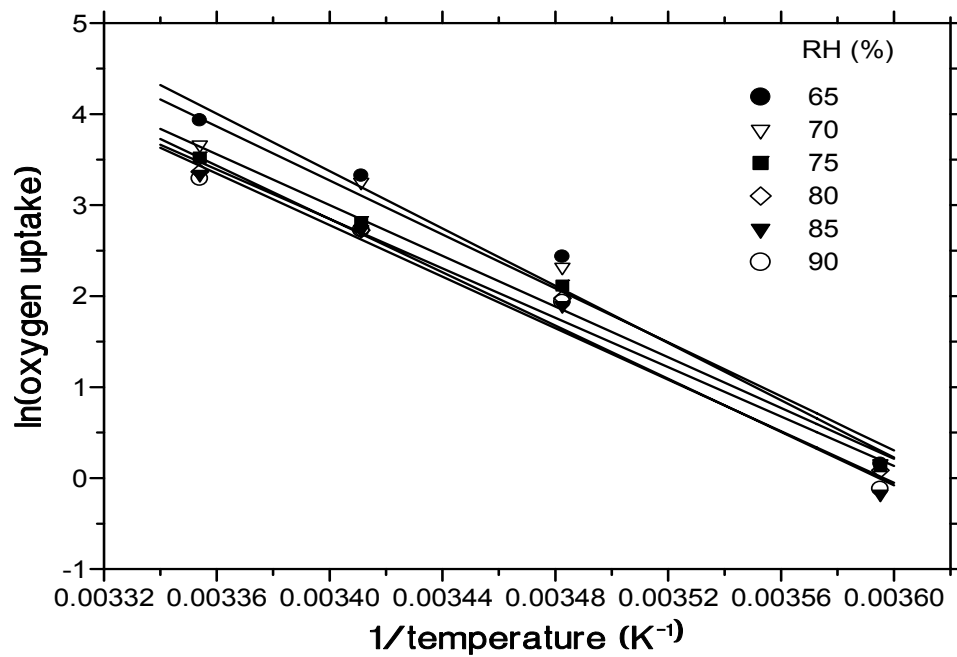


Fig. 2. The Arrhenius model applied to the V_m for oxygen, obtained from the power function. (●) 65% RH, (▽) 70% RH, (■) 75% RH, (◇) 80% RH, (▼) 85% RH and (○) 90% RH.

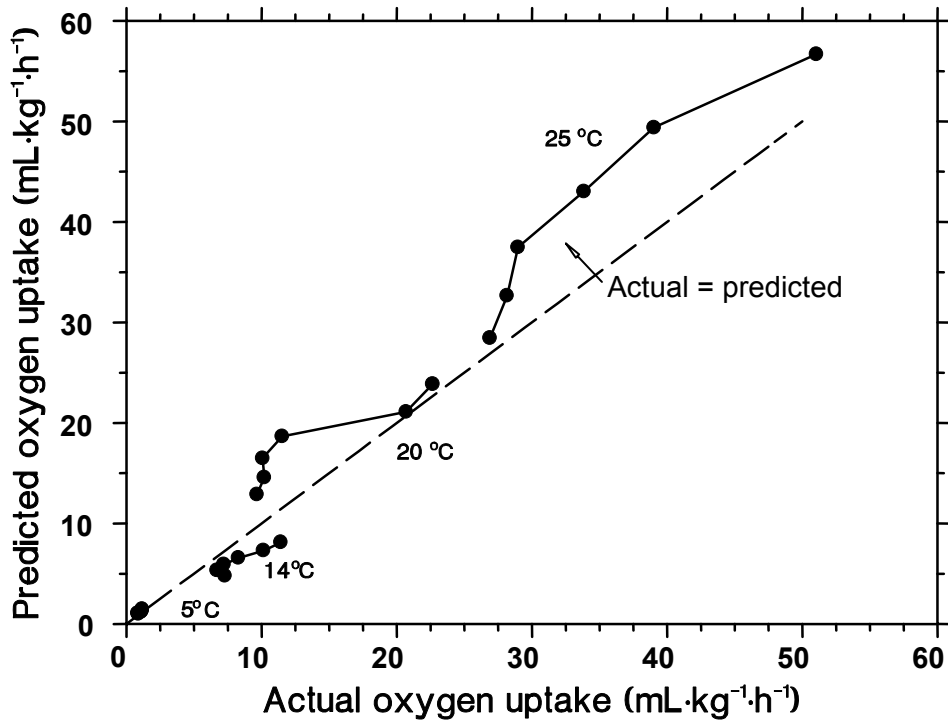


Fig. 3. Actual versus predicted oxygen uptake for prickly pear stems held at 24 combinations of temperature and relative humidity. Equation for modeled respiration:
 $EXP((-0.1985+50.96*(1/(273.15+T))))*RH+67.638-18430*(1/(273.15+T))$.

