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Coupling the dynamics of diffused gases and microbial growth in modified atmosphere packaging

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ABSTRACT

Coupling microbial dynamics with the complete dynamics of the packaging gases is still a challenge. In this work the microbial growth kinetic parameters for *Pseudomonas* and Lactic Acid Bacteria (LAB) in MAP are identified based on accurate estimation of diffusivity of gases and parameter scaled sensitivity approaches. The microbial dynamics are also compared with those estimated based on partial pressure measurement. Scaled sensitivity coefficient analysis using dissolved gases as variable inputs, shows that in most cases the only coefficients large enough for estimation were those for $CO_{2max\text{-}diss}$, and for μ_{max}. The current data showed that dissolved gases led significant differences on the microbial parameter of CO_{2max} values when compared with the headspace gases. On the other hand, the (so-called) dissolved specific growth rate follows a clear trend down for both microorganisms in relation to the increase of the initial headspace CO_2. Finally, current results indicate a possible correlation between $CO_{2max\text{-}diss}$, $CO_{2max\text{-}headspace}$, and μ_{max} as functions of CO_{2init}.

1. Introduction

It is well documented in the literature that the main microbial spoilage microorganisms occurring in freshly cut meat are *Acinetobacter*, *Pseudomonas*, *Brochothrix*, *Flavobacterium*, *Psychrobacter*, *Moraxella*, *Staphylococcus*, *Micrococcus*, lactic acid bacteria (LAB) and genera of the family of Enterobacteriaceae (refer for an extensive review to Doulgeraki et al. (2012)). Amongst these, the most predominant spoilage microorganisms appear to be *Pseudomonas*, *B. thermosphacta*, and lactic acid bacteria. Evidently, the factors which define their levels and overall microbial diversity present include the meat constituents, temperature, pH, packaging conditions (i.e., levels of oxygen or carbon dioxide and competing microbiota (Doulgeraki et al., 2012; Koutsoumanis et al., 2006; Lambert et al., 1991)). In the case of modified atmospheric packaging conditions, high concentrations of CO_2 (up to 10%) have been found to inhibit the growth of most *Pseudomonas* spp. Similar observations were also obtained based on microbiota analysis, by 16S rDNA pyrosequencing, of poultry meat stored in MAP (Rouger et al., 2018). *Ps. fragi* appears as the dominant *Pseudomonas* regardless of the packaging conditions. All the other *Pseudomonas* species mainly occur during air storage of raw meat (Doulgeraki et al., 2012). On the other hand, LAB are important competitors of the other spoilage related microbial groups under MAP conditions (e.g., Nychas and Skandamis (2005)). They have been considered specific meat spoilage organisms in MAP for cold-stored meat products while their microbial diversity has been evaluated in several studies (e.g., Doulgeraki et al. (2010), Vihavainen et al. (2007)).

It has been reported that the bacteriostatic effect of CO_2 within MAP is primarily influenced by CO_2 absorption into the food system (Devlieghere et al., 2001; Meredith et al., 2014). Nevertheless, Estelle Chaix et al. (2015a, 2015b) accurately described that the main bottleneck in the evaluation and study of the impact of O_2/CO_2 transfer on the growth of microorganisms seems to be the quantification of dissolved O_2 and CO_2, especially when dealing with solid fatty foods and not only with liquids. Therefore, it is a common practise that models developed for representing the effect of O_2/CO_2 on the growth of microorganisms usually only consider the headspace content of O_2/CO_2, that is the partial pressure (e.g.,(Alfaro et al., 2013). In the last few years some researchers considered combining both gas transfers through packaging and within food and the impact of these gases on the growth of microorganisms (e.g., Chaix et al. (2015a, 2015b), Guillard et al. (2016)). Nevertheless, it is still of a challenge to couple microbial dynamics with the complete dynamics of the packaging gases and not only the headspace content of O_2/CO_2. In addition, the impact on

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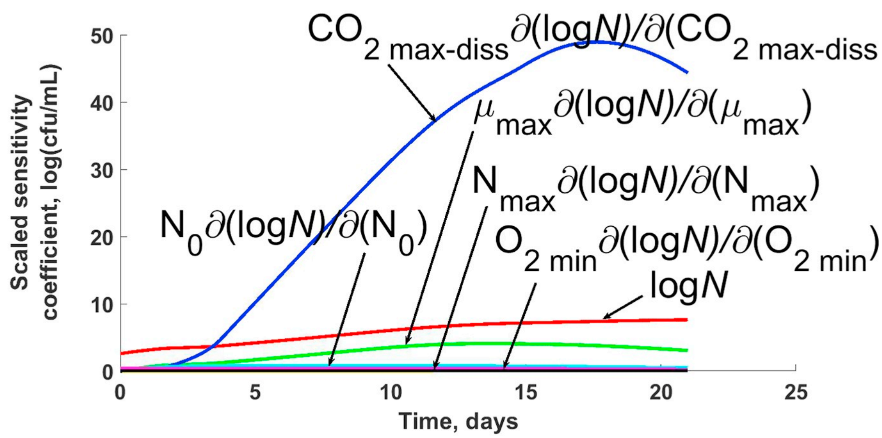

Fig. 1. Example of scaled sensitivity coefficients (SSC) and log$N$(t) for Eq. (6) for *Pseudomonas* growth in 90:10 N_2:CO_2 MAP, using dissolved CO_2 and O_2 gases. SSC for Q(0) not shown because it was very small.

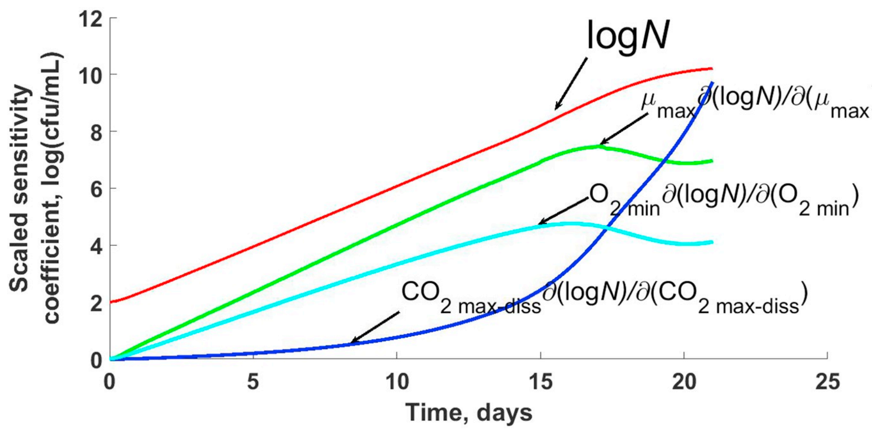

Fig. 2. Example of scaled sensitivity coefficients and log$N$(t) for Eq. (6) for *Pseudomonas* growth in 80:20 N_2:CO_2 MAP, using dissolved CO_2 and O_2 gases. SSCs for O_2, N_{max}, N(0) and Q(0) not shown because they were very small.

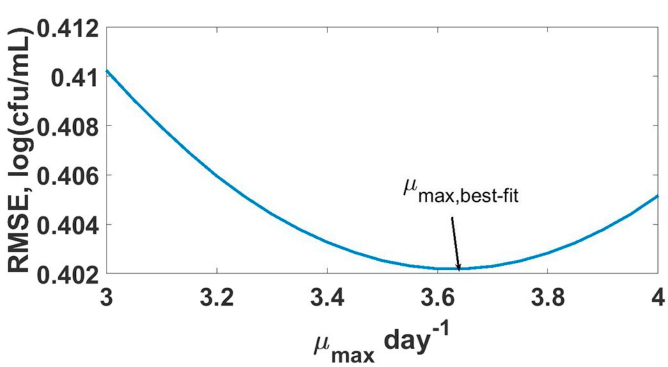

Fig. 3. Example of Root mean square error (RMSE) vs. μ_{max} for *Pseudomonas* growth in 40:30:30 CO_2:O_2:N_2 MAP, using dissolved CO_2 and O_2 gases. At the best-fit value of μ_{max} = 3.63 day^{-1}, the best-fit CO2max-diss value was estimated as 1000 ± 21.95 (mean ± standard error) (Table 1).

estimating microbial growth kinetics in relation to dissolved or headspace gas levels still needs to be unravelled.

When developing dynamic modelling approaches, i.e., taking into account all the gradient changes (e.g., dynamics of O_2 and CO_2 in MAP food), model parameter identification becomes a challenging exercise. Scaled sensitivity coefficients could contribute in parameter identifiability as they can determine whether parameters can be estimated and which parameters will have the smallest relative error (Cattani et al., 2016; Dolan et al., 2013; Dolan and Mishra, 2013). The aim of this work is to estimate microbial kinetic parameters for growth of *Pseudomonas* and Lactic Acid Bacteria (LAB) in MAP (of low permeability) based on accurate estimation of diffusivity of gases and scaled sensitivity parameter approaches. The microbial dynamics are also compared with those estimated based on partial pressure measurement which is still the most common practise in literature.

2. Materials and methods

2.1. Microbial data and gas analysis

Previously published experimental data were used as described in Meredith et al. (2014). In summary skinless chicken fillets were stored in gaseous mixtures of 10%, 30%, 50%, 70% and 90% CO_2 balanced with N_2, 80:20%, O_2:N_2 and 40:30:30% CO_2:O_2:N_2 and control conditions (air) at 2 °C without light exposure. The trays used had bar oxygen transmission rate of of 0.15 cm^2/Pck d and were covered with a 76 mm antifog high barrier film with an oxygen transmission rate of 0.8 ml/m^2/24 h. The choice was made such as the exchange of gases with the external environment is limited. *Pseudomonas* and lactic acid bacteria (LAB) over 21 days of storage are used for the current analysis. The headspace gas composition (O_2 and CO_2 (ml/100 ml)) in all packs was measured immediately after packing and at several sampling time intervals an oxygen and carbon dioxide analyser were used (Checkmate 9900 analyser, PBI-Dansensor, Ringsted, Denmark).

The CO_2 solubility was determined by monitoring the changes in the headspace volume over time using a buoyancy technique and performing calculations based on volumetric measurements and the

Table 1
Parameter estimates for modelling *Pseudomonas* growth using dissolved gases. (For all treatments, C(0) was fixed at 0.5. Both µ_max and CO2_max_diss were estimated. All other parameters were held constant.)

Treatment	Parameter	Estimate	SE	relerr	95% CI		rmse[a]
90:10 N_2:CO_2	µ_max[b]	10.7					0.394
	CO_2_max_diss[c]	255.3	0.98	0.38%	253.03	257.58	
	O_2_min[c]	0.1					
	Nmax[d]	10ˆ8					
	No[d]	10ˆ2.6					
70:30 N_2:CO_2	µ_max	4.15					0.274
	CO_2_max_diss	613.74	3.17	0.52%	606.67	620.81	
	O_2_min	0.1					
	Nmax	10ˆ7					
	No	10ˆ2.6					
50:50 N_2:CO_2	µ_max	4.8					0.290
	CO_2_max_diss	1090	14.89	1.37%	1057	1124	
	O_2_min	0.1					
	Nmax	10ˆ5					
	No	10ˆ2					
30:70 N_2:CO_2	µ_max	1.6					0.576
	CO_2_max_diss	1816.9	75.44	4.15%	1648.8	1984.9	
	O_2_min	0.1					
	Nmax	10ˆ6					
	No	10ˆ2					
10:90 N_2:CO_2	µ_max	1.5					0.612
	CO_2_max_diss	3067.5	175.36	5.72%	2676.8	3458.2	
	O_2_min	0.1					
	Nmax	10ˆ7					
	No	10ˆ2					
40:30:30 CO_2:O_2:N_2	µ_max	3.63					0.402
	CO_2_max_diss	1000	21.95	2.19%	903.5883	971.3285	
	O_2_min	11					
	Nmax	10ˆ7					
	No	10ˆ2					
80:20 O_2:N_2	µ_max	4.58	0.38	8.29%	3.72	5.44	0.710
	CO_2_max_diss	987.72	104.19	10.55%	752.03	1223.41	
	O_2_min	11					
	Nmax	10ˆ11					
	No	10ˆ2					

[a] log(cfu/ml)
[b] day^{-1}.
[c] ppm.
[d] cfu/mL.

Henry's constant. Henry's constant was also used to estimate the oxygen solubility in the chicken fillets. All chicken samples were analysed for *Campylobacter* spp. and lactic acid bacteria (LAB), on Campylobacter blood-free agar base and De Man Rogasa Sharpe agar, respectively (refer to Meredith et al. (2014) regarding all the experimental procedures applied).

2.2. Modelling

2.2.1. Equations

Two modelling approaches were implemented:

- Modelling of the simultaneous effect of O_2 and CO_2 gases on the growth of the studied microorganisms based on the gas concentrations or partial pressures (their equivalent in gaseous headspace) during the storage times.
- Modelling on the simultaneous effect of dissolved O_2 and CO_2 gases on the growth of the studied microorganisms.

The primary model used for the microbial growth consisted of two ordinary differential equations:

$$\frac{dN(t)}{dt} = \mu(Q, CO_2, O_2)\cdot\left(1 - \frac{N(t)}{N_{\max}}\right)\cdot N(t) \quad \text{(1a)}$$

$$\frac{dQ(t)}{dt} = \mu_{\max}\cdot Q(t) \quad \text{(1b)}$$

where

$$\mu(Q, CO_2, O_2) = \mu_{\max}\cdot\mu_Q(Q)\cdot\mu_{CO_2}(CO_2)\cdot\mu_{O_2}(O_2) \quad (2)$$

$N(t)$ is microbial concentration (cfu/mL)$Q(t)$ has to do with microbial physiology (cfu/mL)µ (Q, CO2, O2) is the specific growth rate (day − 1), dependent on Q, CO2 and O2.

The secondary models were:

$$\mu_{CO_2}(CO_2) = \left(\frac{CO_{2\ max-diss} - CO_2(t)}{CO_{2\ max-diss}}\right) \quad (3)$$

$$\mu_{O_2}(O_2) = \left(\frac{O_{2\ min}}{O_2(t) + O_{2\ min}}\right) \quad (4)$$

$$\mu_Q(Q) = \left(\frac{Q(t)}{1 + Q(t)}\right) \quad (5)$$

The integrated form of the model for Eq. (1a) was found by substituting Eqs. (3), (4), and (5) into Eq. (2), and by substituting Eq. (2) into Eq. (1a):

$$\frac{dN(t)}{dt} = \mu_{\max}\left(\frac{Q(t)}{1 + Q(t)}\right)\left(\frac{CO_{2\ max-diss} - CO_2(t)}{CO_{2\ max-diss}}\right) \cdot\left(\frac{O_{2\ min}}{O_2(t) + O_{2\ min}}\right)\left(1 - \frac{N(t)}{N_{max}}\right)\cdot N(t) \quad (6)$$

The parameters of the full model in Eqs. (6) and (1b) were:

Table 2
Parameter estimates for modelling *Pseudomonas* growth using headspace CO_2 and headspace O_2. (For all treatments, C(0) was fixed at 0.5. Both μ_max and CO2_max_diss were estimated. All other parameters were held constant.)

Treatment	Parameter	Estimate	SE	relerr	95% CI		rmse[a]
90:10 N_2:CO_2	μ_max[b]	3.05	0.25	8.18%	2.49	3.61	0.3656
	CO_2_max_diss[c]	14.75					
	O_2_min[c]	3					
	Nmax[d]	10ˆ7.5					
	No[d]	10ˆ2.6					
70:30 N_2:CO_2	μ_max	1.45					0.2842
	CO_2_max_diss	57.6613	4.71	8.17%	47.17	68.15	
	O_2_min	0.5					
	Nmax	10ˆ7					
	No	10ˆ2.6					
50:50 N_2:CO_2	μ_max	13					0.3046
	CO_2_max_diss	51.9384	1.38	2.66%	48.86	55.02	
	O_2_min	0.25					
	Nmax	10ˆ5					
	No	10ˆ2					
30:70 N_2:CO_2	μ_max	2					0.5948
	CO_2_max_diss	142.1949	23.26	9037.8%	194.01	169.12	
	O_2_min	0.1					
	Nmax	10ˆ6					
	No	10ˆ2					
10:90 N_2:CO_2	μ_max	2					0.6089
	CO_2_max_diss	196.23	24.26	12.4%	142.18	250.29	
	O_2_min	1.8					
	Nmax	10ˆ7					
	No	10ˆ2					
40:30:30 CO_2:O_2:N_2	μ_max	1.525					0.5258
	CO_2_max_diss	119.4395	25.94	21.72%	61.63	177.25	
	O_2_min	26					
	Nmax	10ˆ7					
	No	10ˆ2					
80:20 O_2:N_2	μ_max	4.8					0.3868
	CO_2_max_diss	58.2026	5.07	8.72%	46.9	69.5	
	O_2_min	20					
	Nmax	10ˆ11					
	No	10ˆ2.6					
control	μ_max	22.5					0.291
	CO_2_max_diss	18.5713	0.12	0.62%	18.3	18.8	
	O_2_min	1.2					
	Nmax	10ˆ11					
	No	10ˆ2.6					

[a] log(cfu/ml).
[b] day^{-1}.
[c] ppm.
[d] cfu/ml.

μ_{max} Maximum specific growth rate, day^{-1};

$CO_{2\ max-diss}$ Maximum concentration (ppm) of dissolved CO_2 that permits growth;

$O_{2\ min}$ Minimumum concentration (ppm) of O_2 required for growth;

N_{max} Maximum concentration (CFU/mL) of microorganisms at long time;

N_0 Initial concentration(CFU/mL) of microorganisms;

$Q(0)$ Initial value having to do with the microbial physiology.

The lag phase λ (hr) is implicitly incorporated into $Q(0)$ as follows: $h_0 = \mu_{max} * \lambda$; $q_0 = 1/(\exp(h_0)\text{-}1)$; $Q(0) = \ln(q_0)$ (Gumudavelli et al., 2007). In this work, we used $Q(0)$ directly as a parameter, and did not deal with the individual values of h_0, λ, and q_0.

When headspace gases were used, the parameter $CO_{2max\text{-}diss}$ name was changed to $CO_{2max\text{-}headspace}$. For dissolved gases, the values of $CO_2(t)$ dissolved and $O_2(t)$ dissolved were obtained by interpolating from the experimental values. Similarly, for the headspace gases, the values of $CO_2(t)$ headspace and $O_2(t)$ headspace were obtained by interpolating from the experimental values.

2.2.2. Estimation of model parameters

Seven experimental conditions plus a control (originating from the work of Meredith et al. (2014)) were analysed while two microbial concentrations, *Pseudomonas* (Ps) and *lactic acid* (LA), were used for parameter estimation. The variables that were used as inputs were: 1) Dynamic concentration of dissolved gases CO_2, O_2, and N_2; and 2) Dynamic concentration of headspace gases CO_2, O_2, and N_2. Therefore, the total number of sets of parameters were 7 conditions × 2 dependent variables, x 2 variables = 28.

To estimate the parameters for each of the 28 sets, an inverse problem solution method was needed. Briefly, a parameter can be estimated only if the scaled sensitivity coefficient is *large* compared to the dependent variable, and is *uncorrelated* with all other parameters (Dolan and Mishra, 2013). The sensitivity of the model with respect to each parameter is found by taking the first derivative of the model (logN) with respect to each parameter. The sensitivity is *scaled* by multiplying by the parameter, to obtain the same units as logN. Then all scaled sensitivities can be plotted together with the dependent variable on one chart and compared.

The parameter estimation problem was solved as follows:

Table 3
Parameter estimates for modelling lactic acid bacteria growth using dissolved CO_2 and dissolved O2 gases. (For all treatments, C(0) was fixed at 0.5. Both μ_max and CO2_max_diss were estimated. All other parameters were held constant.)

Treatment	Parameter	Estimate	SE	relerr	95%CI		rmse[a]
90:10 N_2:CO_2	μ_max[b]	7.35					0.4107
	CO_2_max_diss[c]	262.2961	1.30	259.41	265.18	11.57	
	O_2_min[c]	0.08					
	Nmax[d]	10ˆ7.5					
	No[d]	10ˆ1.67					
70:30 N_2:CO_2	μ_max	3.4					0.2333
	CO_2_max_diss	678.0985	5.25	0.77%	666.40	689.80	
	O_2_min	0.08					
	Nmax	10ˆ6.5					
	No	10ˆ1.67					
50:50 N_2:CO_2	μ_max	3.4					0.2331
	CO_2_max_diss	1130.2	8.10	0.72%	1112.15	1148.25	
	O_2_min	0.1					
	Nmax	10ˆ6					
	No	10ˆ1.67					
30:70 N_2:CO_2	μ_max	0.5					0.2244
	CO_2_max_diss	2755.3	132.83	4.82%	2459.32	3051.25	
	O_2_min	0.9					
	Nmax	10ˆ5.5					
	No	10ˆ1.6					
10:90 N_2:CO_2	μ_max	0.7					0.2341
	CO_2_max_diss	4240	285.52	6.73%	3603.79	4876.15	
	O_2_min	0.1					
	Nmax	10ˆ5					
	No	10ˆ1.6					
40:30:30 CO_2:O_2:N_2	μ_max	1.4					0.2422
	CO_2_max_diss	2929.7	317.40	10.83%	2222.46	3637.01	
	O_2_min	10					
	Nmax	10ˆ6.5					
	No	10ˆ1.6					
80:20 O_2:N_2	μ_max	2.6					0.3087
	CO_2_max_diss	1084.5	71.47	6.59%	925.3	1243.8	
	O_2_min	26					
	Nmax	10ˆ7					
	No	10ˆ1.6					
Control	μ_max	1.1					0.1322
	CO_2_max_diss	1028.8	84.17	8.18%	841.3	1216.4	
	O_2_min	6					
	Nmax	10ˆ6.5					
	No	10ˆ1.6					

[a] log(cfu/ml).
[b] day^{-1}.
[c] ppm.
[d] cfu/ml.

1 Solutions to Eq. (1a) were converted to $\log_{10}(N(t))$, because the error variance over time was nearly constant, as is the case for most microbial predictive modelling;

To determine which of the 6 parameters could be estimated, the scaled sensitivity coefficients (SSCs) were plotted using nominal values of the parameters, based on the experimental conditions. Each parameter β_i had its own SSC (Dolan and Mishra, 2013):

$$\beta_i \frac{\partial(\log N(t))}{\partial \beta_i} \quad (7)$$

2 For each of the 28 conditions, 6 SSCs (one for each parameter) and $\log N(t)$ were drawn on the same plot. Any parameter with an SSC maximum absolute value that was too small (less than 5% of the logN(t) span) was set to a constant. That parameter could not be estimated, because the model was insufficiently sensitive to that parameter.

Any parameter with an SSC that was highly correlated to another SSC could not be estimated simultaneously with the other parameter. To estimate both parameters, one correlated parameter (β_A) was set to different constant values, and the other parameter (β_B) was estimated for each fixed β_A. The root mean square error (RMSE = $\sqrt{SSE/(n-p)}$, where SSE = sum of squares of errors; n = number of data; p = number of parameters) was plotted vs. β_A and the minimum RMSE was found. The values of β_A and β_B at the RMSE minimum were the final.

3 From the results of #2, the identified parameters were estimated for each of the 28 conditions. The nonlinear regression function nlinfit in MATLAB (version 2017a) and ode45 were used. Parameter errors were computed as the square root of the diagonal of the parameter variance-covariance matrix. Correlations between any two parameters were given from the correlation matrix, ($r12 = \sigma_{12}/(\sigma_1 * \sigma_2)$, where σ_1 and σ_2 are the parameter 1 and parameter 2 errors, and σ_{12} is the covariance of parameter 1 and parameter 2, which is in the off-diagonals for the variance-covariance matrix (Dolan, 2003). Correlation was also determined visually by comparing the shapes of the SSCs. If any two parameters had SSCs with similar shapes, then they were highly correlated.

3. Results and discussion

The SSC plot for *Pseudomonas* in 90:10 N_2:CO_2, using dissolved gases as variable inputs, shows that the only SSCs large enough for

Table 4

Parameter estimates for modelling lactic acid bacteria growth using headspace CO_2 and headspace O_2. (For all treatments, C(0) was fixed at 0.5. Both μ_max and CO2_max_diss were estimated. All other parameters were held constant.)

Treatment	Parameter	Estimate	SE	relerr	95%CI		rmse[a]
90:10 N_2:CO_2	μ_max[b]	11					0.6068
	CO_2_max_diss[c]	11.3111	0.12	1.04%	11.05	11.57	
	O_2_min[c]	0.3					
	Nmax[d]	10ˆ7					
	No[d]	10ˆ1.6					
70:30 N_2:CO_2	μ_max	2					0.2454
	CO_2_max_diss	74.714	5.81	7.78%	61.77	87.66	
	O_2_min	0.5					
	Nmax	10ˆ7					
	No	10ˆ1.6					
50:50 N_2:CO_2	μ_max	21.6					0.1856
	CO_2_max_diss	46.1736	0.23	0.50%	45.66	46.69	
	O_2_min	0.25					
	Nmax	10ˆ6					
	No	10ˆ1.6					
30:70 N_2:CO_2	μ_max	6.6					0.1655
	CO_2_max_diss	67.4937	0.38	0.56%	66.65	68.34	
	O_2_min	0.9					
	Nmax	10ˆ5.5					
	No	10ˆ1.6					
10:90 N_2:CO_2	μ_max	7					0.1886
	CO_2_max_diss	93.0403	0.67	0.72%	91.54	94.54	
	O_2_min	1					
	Nmax	10ˆ5					
	No	10ˆ1.6					
40:30:30 CO_2:O_2:N_2	μ_max	3					0.2253
	CO_2_max_diss	46.7967	0.81	1.73%	44.99	48.60	
	O_2_min	26					
	Nmax	10ˆ7					
	No	10ˆ2					
80:20 O_2:N_2	μ_max	2					0.3259
	CO_2_max_diss	98.4767	9.51	9.66%	77.3	119.7	
	O_2_min	26					
	Nmax	10ˆ7					
	No	10ˆ1.6					
Control	μ_max	28.2					0.1328
	CO_2_max_diss	18.7292	0.18	0.94%	18.3	19.1	
	O_2_min	0.5					
	Nmax	10ˆ6.5					
	No	10ˆ1.6					

[a] log(cfu/ml).
[b] day^{-1}.
[c] ppm.
[d] cfu/ml.

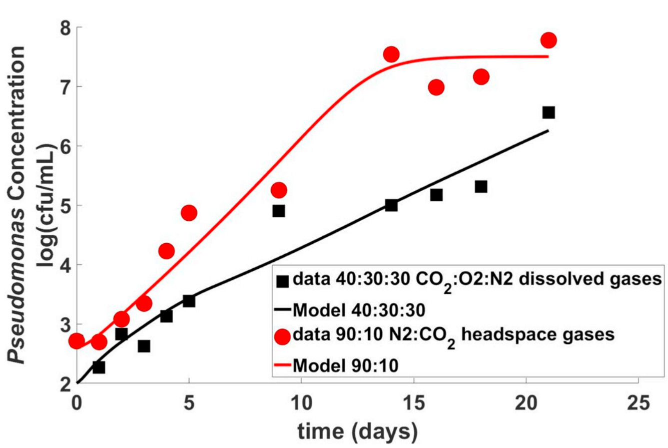

Fig. 4. Log of *Pseudomonas* concentration versus time for 40:30:30 CO_2:O_2:N_2 MAP, using dissolved CO_2 and O_2 gases. Log of *Pseudomonas* concentration versus time for 90:10 N_2:CO_2 MAP, using headspace CO_2 and O_2 gases. Markers are experimental values. The lines are the fitted plots, based on the estimated parameters in Table 1.

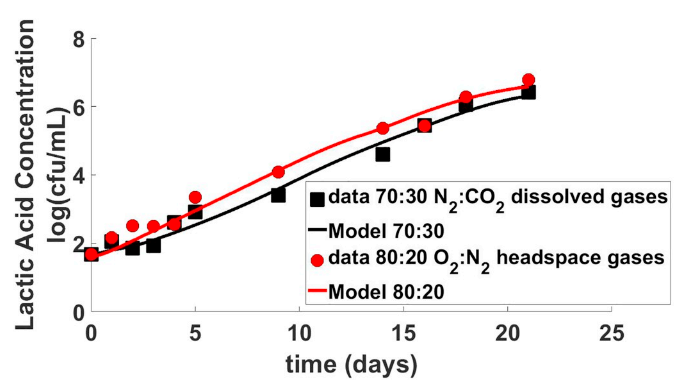

Fig. 5. Log of lactic acid bacteria concentration versus time for 70:30 N_2:CO_2 MAP, using dissolved CO_2 and O_2 gases. Log of lactic acid bacteria concentration versus time for 80:20 O_2:N_2 MAP, using headspace CO_2 and O_2 gases. Markers are experimental values. The lines are the fitted plots, based on the estimated parameters in Tables 2 & 3.

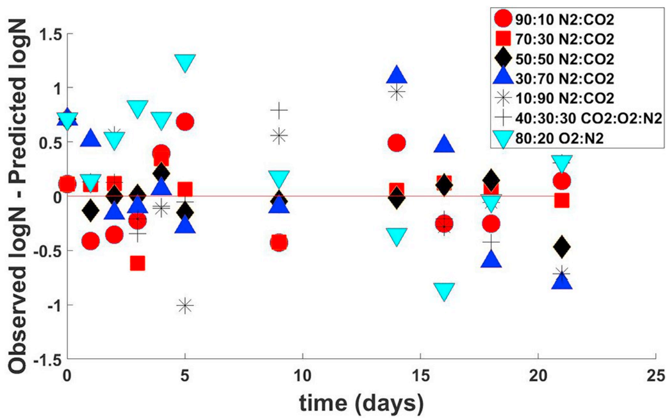

Fig. 6. Residual scatter plot for all seven conditions for *Pseudomonas* growth model using dissolved gases.

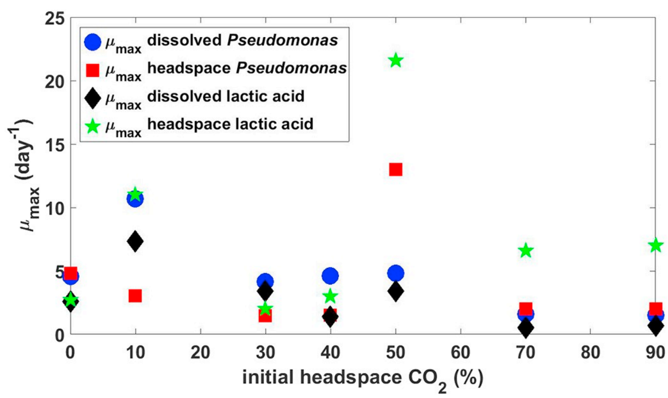

Fig. 7. Summary of μ_{max} versus initial headspace CO_2 based on dissolved gases and based on headspace gases for *Pseudomonas* and for lactic acid bacteria. The μ_{max} values are taken from Tables 1–4.

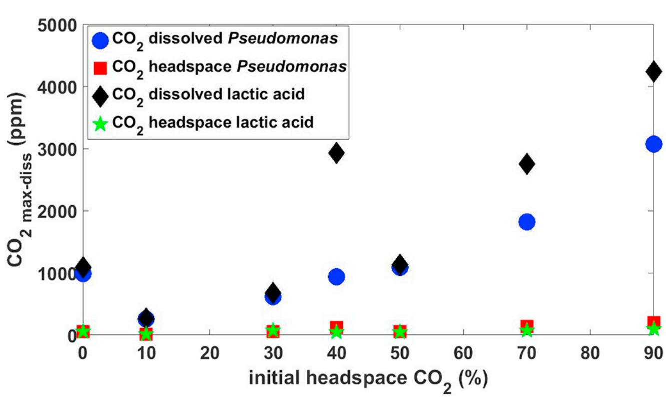

Fig. 8. Summary of $CO_{2max\text{-}diss}$ versus initial headspace CO_2 based on dissolved gases and based on headspace gases for *Pseudomonas* and for lactic acid bacteria. The $CO_{2max\text{-}diss}$ values are taken from Tables 1–4.

estimation were those for $CO_{2max\text{-}diss}$, and for μ_{max} (Fig. 1). The SSC plot for *Pseudomonas* in 80:20 N_2:CO_2 shows that the parameters $CO_{2max\text{-}diss}$, μ_{max}, and O_{2min} were all large enough for estimation (Fig. 2). However, μ_{max} and O_{2min} were correlated (similar shapes), and could not be estimated separately. For the lactic acid bacteria experiments, the SSC plots (not shown) showed that only two parameters, $CO_{2max\text{-}diss}$, and μ_{max} had SSCs that were large enough for estimation.

Most of the SSC plots were similar to that in Fig. 1. Since $CO_{2max\text{-}diss}$ was always the largest SSC, and was usually correlated with μ_{max}, μ_{max} was set to numerous constant values, $CO_{2max\text{-}diss}$ was estimated, and the best-fit values of $CO_{2max\text{-}diss}$ and μ_{max} were determined at the minimum RMSE (Fig. 3). This procedure shown in Fig. 3 was done for both *Pseudomonas* and for lactic acid bacteria.

The parameter values for all 28 experimental conditions are shown in Tables 1–4. RMSE values were on the order of 2–5% of the total logN scale, showing a reasonably good fit. Parameter errors on CO2max-diss were less than 10% in all cases.

Example fits are shown for each of the four cases in Figs. 4–5. Residuals plot (see example in Fig. 6) and residual histogram analysis (not shown) for all seven fits of *Pseudomonas* using dissolved gases show that the residuals were reasonably additive, nearly zero mean, Gaussian, and had constant variance. The concave-down shape of the residuals indicate that there was some correlation in the residuals, suggesting that the model could be improved.

The trends of the two estimated parameters with the initial CO_2 headspace gas ($CO_{2\ init}$) are shown in Figs. 7 and 8 for *Pseudomonas* and for lactic acid bacteria. As expected, $CO_{2max\text{-}diss}$ increased much and $CO_{2max\text{-}headspace}$ increased some with $CO_{2\ init}$ while μ_{max} decreased with CO_{2init}. The responses of the CO_{2max} and μ_{max} are different when the dissolved or the headspace gases are considered. Interestingly, Estelle Chaix et al. (2015a, 2015b) reviewed the sensitivity of CO_{2max} of microorganisms based on a number of previous studies which lead to erroneous findings in which even 100% of CO_2 in the headspace would not be enough to inhibit bacterial growth.

There was an unexpected increase in μ_{max} only for headspace gases at only 50% initial CO_2 for both *Pseudomonas* and for lactic acid bacteria. It is not clear if this large increase is due to some synergistic effect of having equal concentrations of N_2 and O_2, or is due to rapidly increased inactivation sensitivity of the microorganisms at these levels. Future experiments with more data near the 50:50 levels will need to be done.

These observations highlighted the importance of working in the dissolved or headspace gases when assessing the microbial kinetics. The current data showed that dissolved gases led to significant differences on the CO_{2max} values. On the other hand the specific growth rate follows a clear trend in relation to the increase of the initial headspace CO_2. Finally, current results indicate a possible correlation between $CO_{2max\text{-}diss}$, $CO_{2max\text{-}headspace}$, and μ_{max} as functions of CO_{2init}. If secondary models showing these 3 parameters as functions of CO_{2init} could be validated, it would be a big step toward generalizing the effects of gas dynamics on microbial growth in MAP products. Such models could help researchers and companies do what-if scenarios for MAP products, more accurately design experiments, and reduce the number of experiments needed.

4. Conclusions

As previously discussed by Estelle Chaix et al. (2015a, 2015b) the integration of the gas impact in the predictive models can allow prediction of the microbial growth and at the same time the gas production/consumption that can have an impact on O_2/CO_2 content in the headspace. Models developed for representing the effect of O_2/CO_2 on the growth of microorganisms usually only consider the headspace content of O_2/CO_2. In this study, the effect of O_2 concomitantly with that of CO_2 on the microbial growth kinetics was taken into account for both the dissolved and the headspace content of the gases present in the package.

Future studies should focus on quantitative assessment of spatial bacteria distribution to study the influence of the impact of O_2 gradient on the growth modelling the spatial dynamic of CO_2 transfer and its impact on local values of pH (Estelle Chaix et al., 2015a, 2015b). Other studies could also quantitatively assess the dependence of gas solubility on product temperature.

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