EVALUATION OF THE FERMENTABILITY OF OAT FRACTIONS OBTAINED BY DEBRAINING USING LACTIC ACID BACTERIA

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ABSTRACT

Aims: The overall kinetics of the fermentation of four oat fractions obtained by debranning using three potentially probiotic lactic acid bacteria were investigated. The main objective was to study the suitability of these fractions as fermentation media for the growth and the metabolic production of bacteria isolated from human intestine.

Methods and Results: The cell growth, lactic acid production and substrate uptakes of the three lactobacilli was monitored for 30 hours. An unstructured mathematical model was used to describe and fit the experimental data. In the medium from fraction B (1-3% pearlings or β-glucan rich fraction) all strains reached the highest cell populations, maximum growth rates and maximum lactic acid productions. This could be due to the high levels of total fiber and β-glucan of this fraction. Limited growth and lactic acid formation was found in medium A (0-1% pearlings or bran rich fraction).

Conclusions: Medium B (1-3% pearling fraction) is the most suitable for fermentation and produces considerably higher probiotic cell concentrations.

Significance and Impact of the Study: Debranning technology could be used to separate fractions from cereal grains for the production of functional formulations with higher probiotic levels than the ones that obtained with the whole grain.

Keywords: lactobacillus, probiotic, cereal, fermentation, debranning, prebiotic, unstructured mathematical model.

INTRODUCTION

A common characteristic of the human gut microflora is their high fermentative capacity (Cummings and MacFarlane 1991; MacFarlane *et al.* 1992a; Gibson and MacFarlane 1995). The anaerobic breakdown of substrates, such as undigested polysaccharides, resistant starch, and fibre, enhances the formation of lactic acid bacteria, but also of short-chain fatty acids as fermentation products (Ruppin 1980; Bailey and Ollis 1986; MacFarlane *et al*. 1992b; Hirayama 2002). Increased production of short-chain fatty acids leads to a decrease in the pH of the colon and low pH in stool was associated with a reduced incidence of colon cancer in various populations (Caplice and Fitzgerald 1999). Depending on the nature, quantity, and fermentability of indigestible polysaccharides reaching the colon, the proportion of the short-chain fatty acids acetate, propionate, and butyrate can vary. Resistant starch and wheat bran favour the production of butyrate, whereas pectin leads to a higher formation of acetate (Charalampopoulos *et al.* 2002).

Cereal grains contain all of the essential nutrients, fibre, carbohydrates, proteins, vitamins, lipids and minerals. These components are found in specific parts of the grain and are not distributed uniformly. Cereal grains are made up of different layers, and in a simplified way it can be considered made of three parts: bran, endosperm and germ. The endosperm, which contains mostly starch, is the largest fraction and comprises approximately 82 % of a grain (dry basis). The embryo, which is the portion of the grain that develops into the roots and shoot, has the majority of the grain lipids, fats and sugars (Wang 1999). The bran protects the cereal contents and contains high levels of fibre, potassium, sodium, magnesium and calcium (MacMasters *et al.* 1971). The bran layer is

also major source of proteins which is mainly due to the aleurone layer. The aleurone layer contains niacin, phytic acid and phosphorus, and is an excellent source of amino acids for microbial fermentations. However, due to the thick indigestible cell walls of the bran fraction the vitamins and proteins are not easily accessible when ingested by humans. Debranning technology can be used to concentrate aleurone layer nutrients in a fraction rich in fibre and potentially prebiotic oligosaccharides.

In this work, the fermentations of different layer of oat grains obtained through debranning with potentially probiotic lactic acid bacteria will be monitored. Cell growth, metabolic product formation and substrate uptake will be measured and the results will be fitted to an unstructured mathematical model. The significant kinetic parameters obtained will allow the characterisation of these bioprocesses and will be a preliminary step in the formulation of novel potentially prebiotic products.

MATERIALS AND METHODS

Bran removal through debranning

Debranning, also known as pearling, is a process in which the grain layers are sequentially removed by the combined action of friction and abrasion (Wang et al. 2007). Debranning of winter oat grains (naked expression) was carried out using the Satake Abrasive Test Mill Model TM05C. The effect of debranning time on the percentage of bran removal is shown in figure 1. The pearling fractions obtained after 5, 20, and 35 s represent 0-1%, 1-3%, and 3-4.5% of the kernels and are called fractions A, B and C

respectively. Fraction D is the grain left after 35 s and represent the 4.5-100% of the grains.

Fermentation monitoring

Microorganisms and inocula

L. reuteri (NCIMB 11951), *L. plantarum* (NCIMB 8826) and *L. acidophilus* (NCIMB 8821) originally isolated from human intestine were used for the fermentation of the oat fractions. All the lactobacilli strains were stored on slopes of MRS at 4°C.

To obtain sufficient cells for parallel experiments each inoculum was proliferated from the slopes twice in universal bottles containing 20 mL MRS suspension. After 48 h, 0.5 mL of the broth from the first incubation were transferred into freshly sterilized MRS suspension to propagate for another 24 h.

Fermentation procedures

Shake-flask fermentations were performed in duplicate using 500 mL screw-capped glass bottles. In all fermentations, 5% w/v suspensions of the different fractions were prepared and autoclaved at 121°C for 15 min. Bottles were inoculated with a 2% v/v of lactic acid bacteria and incubated at 150 rpm and 37°C for 30 h. Samples were regularly taken for total cell counting and centrifuged fermented media (10 min, 4500 rpm) were stored at -20°C for later analysis. Fermentations were carried out in duplicate.

Cell enumeration

Viable cells were enumerated using the method of Miles and Misra (Collins 1984). Decimal dilutions of fermentation broths were prepared using sterile Ringer's solution. 12 μL were dropped onto 3-4 day old MRS agar plates and then incubated at 37°C for 2-3 days. Viable cell counts were calculated as $\log_{10}$ colony forming units per mL. Dilutions with less than 10 or more than 130 colonies were discarded.

Chemical analyses

Total dietary fibre, soluble fibre and insoluble fibre were determined according to the method of Prosky *et al.* (1992). β-glucan was measured by the McCleary and Codd method (McCleary and Codd 1991) using an assay kit from Megazyme. The concentration of soluble free amino nitrogen (FAN) during fermentation was assayed by the EBC-ninhydrine colorimetric method (European-Brewery-Convention 1973). The protein content was calculated by multiplying the total Kjeldahl nitrogen by a factor of 6.25. Total reducing sugar (TRS) was assayed by the dinitrosalicylic acid method (Miller 1959), and the concentration of lactic acid was obtained using an analytical kit from Megazyme.

Mathematical models

In order to describe and compared the kinetics of the lactic acid bacteria on the oat fraction media, an unstructured mathematical model was used (Vázquez and Murado 2008; Vázquez and Murado, in press). The variables fitted by this approach were the biomass concentration (X: as $\log_{10}C$, being C the colony forming units per mL), the lactic

acid concentration (L) and the total the reducing sugars (S). The definition and units of the model parameters and variables are shown in table 1.

$$X = \frac{X_m}{1+\exp\left[2+\frac{4\cdot v_{mx}}{X_m}\cdot\left(\lambda_x - t\right)\right]} \quad (1)$$

$$L = \frac{L_m}{1+\exp\left[2+\frac{4\cdot v_{ml}}{L_m}\cdot\left(\lambda_l - t\right)\right]} \quad (2)$$

$$S = S_0 + \frac{X_0}{Y_{x/s}} - \frac{1}{Y_{x/s}}\cdot\frac{X_m}{1+\left(\frac{X_m}{X_0}-1\right)\cdot e^{-\frac{4\cdot v_{mx}}{X_m}\cdot t}} - \frac{m_s\cdot X_m^2}{4\cdot v_{mx}}\cdot\ln\left[\frac{X_0\cdot\left(e^{\frac{4\cdot v_{mx}}{X_m}\cdot t}-1\right)+X_m}{X_m}\right] \quad (3)$$

Numerical and statistical methods

Fitting procedures and parametric estimations calculated from the results were carried out by minimisation of the sum of quadratic differences between observed and model-predicted values, using the non linear least-squares (quasi-Newton) method provided by the macro 'Solver' of the Microsoft Excel XP spreadsheet. Statistica 6.0 software (StatSoft, Inc. 2001) was used to evaluate the significance of the estimated parameters by fitting the experimental values to the proposed mathematical models, and the consistency of these equations.

RESULTS

Chemical composition of oat fractions

150 The chemical composition of the different oat fractions obtained by debranning is shown
151 in table 2. A high fibre and ß-glucan concentration is observed in fraction B (1-3%
152 pearling) which is probably due to the presence of aleurone cells in this fraction. Fraction
153 C (3-4.5% pearling) and fraction D (4.5-100%) contain mostly starch and less fibre.
154
155 **Growth and metabolism of *L. plantarum* in the oat fermentation media**
156 Figure 2 shows the growth of *L. plantarum* and the chemical changes during fermentation
157 in the four media with time. The numerical values of the kinetic parameters obtained
158 from fitting the experimental data to the unstructured mathematical models as well as
159 their corresponding statistical analysis are summarised in the table 3. According to these
160 results, the medium prepared from fraction B (1-3% pearlings) led to the highest
161 maximum biomass production ($P < 0.05$) and the maximum growth rate (v_{mx}). In broth A
162 the cell concentration through fermentation only increased from 5.3 to 6.7 $\log_{10}$ CFU/mL,
163 which is justified by the fact that this fraction mostly contains dead cells and pericarp
164 layer, and there are not enough nutrients for the *Lactobacillus* to grow. The initial
165 concentration of total reducing sugar is very low in this fraction (1.2 g/L), which is
166 consumed very quickly during the fermentation.
167
168 The pH was also monitored and decreased accordingly to the lactic acid formation. The
169 highest concentration ($P < 0.05$) and maximum production rate of this organic acid (v_{ml})
170 was obtained in medium B (1.8 g/L and 0.13 g L^{-1} h^{-1} respectively). The sugar
171 consumption is adequately described by the proposed equation (see statistical analysis in
172 table 3). However, media from fractions A, C and D generated the best yields ($Y_{x/s}$) for

biomass production on TRS ($P < 0.05$). In all fermentations a small initial FAN increment was observed, after which a constant decay was noted. For example in medium B, obtained from the 1-3% pearling fraction, FAN increases first from 79 to 86 mg/L to then decrease to 59.5 mg/L after 30 h.

Growth and metabolism of *L. reuteri* and *L. acidophilus* in oat fermentation media

In Figures 3-4 and tables 4-5 the results obtained for *L. reuteri* and *L. acidophilus* are shown. Very similar trends are observed here when compared with *L. plantarum*. The maximum cell concentration was observed in fraction B ($P < 0.05$) after approximately 12 h (asymptotic phase) with values of 9.2 and 9.4 $\log_{10}$ CFU/mL for *L. reuteri* and *L. acidophilus*, respectively. Fractions B and C also show the highest growth rates (v_{mx}). The evolution of pH followed trends similar to *L. plantarum* with higher pH drops noted in media B, C and D. The maximum lactic acid concentration (L_m) for *L. acidophilus* in the broth B was the highest (1.8 g/L). *L. reuteri* in all fractions gave lower maximum lactic acid concentrations than *L. acidophilus*. For the two strains tested the lactic acid production in the A broth was not very significant, with L_m values of 57 mg/L. In both cases the final TRS concentrations after 30 h decreased to 0.6 g/L, 3 g/L, 2.6-2.9 g/L and 2.3-2.7 g/L in the A, B, C and D fractions, respectively. The maximum values of $Y_{x/s}$ were obtained in broths A, B and C.

DISCUSSION

Due to the complexity of cereal substrates, the main compositional changes that were monitored to justify their fermentability were FAN and TRS. Research studies using semi-defined synthetic media have identified these compounds as the most crucial factors for LAB growth (Taillandier *et al.* 1996; Loubiere *et al.* 1997). Moreover, oat grains generally contain significant amounts of minerals, such as manganese and magnesium, and vitamins of the B-complex (Bock 1991). It must be noted that the observed differences in the amount of FAN and sugars present in the cereal media prior to inoculation could be attributed to browning reactions between free amino acids and sugars taking place during the sterilization process. However, the error bars of mean cells values of the two replicate fermentations were in all cases smaller than 0.4 $\log_{10}$ CFU/mL.

Growth of lactobacilli in the oat fraction broths

All of the Lactobacilli strains grew well in broths from fractions B, C and D. The growth in broth A was very low since this medium is made from an oat fraction that contains the outer layers of the kernel with dead cells, small parts of the pericarp and few nutrients. In the four fractions investigated *L. plantarum* achieved a slightly higher viable cell concentration while *L. reuteri* showed the lowest. Broths B, C and D gave viable cell levels above the minimum probiotic requirement of 10^6 CFU/mL (Sanders and Veld 1999).

L. plantarum isolated from togwa has been reported to grow in a cereal-based medium (sorghum-maize gruel, 10% w/v) up to of 9.0 $\log_{10}$ CFU/mL with a final pH of 3.24

(Mugula *et al.* 2003). Marklinder and Lonner (1992; 1994) also investigated the growth of the probiotic *L. plantarum* 299v in an oatmeal medium (18.5 % w/w). In this case the microbe grew well and the final pH dropped to 4.1. This is in broad agreement with the results of this work which achieved similar viable cell numbers and pH values with the intestinal isolate *L. plantarum* (NCIMB 8826).

Oats, unlike other cereals, have received considerable interest as delivery vehicles for probiotics due to their high content of soluble and insoluble fibres resulting in positive effects on blood cholesterol levels (Angel *et al.* 2006; Martensson *et al.* 2001). As a result, many lactic acid bacteria have been used to ferment oat-media including the probiotic cultures *L. reuteri* ATCC 55730 and *L. acidophilus* DSM 20456. These cultures were able to achieve viable cell numbers of 8.0 and 7.3-8.0 $\log_{10}$ CFU/mL and a pH of 3.0 and 4.4 respectively (Martensson *et al.* 2002). Lupine (a legume similar in composition to some cereals) has also proved to be able to support the growth of various strains of *L. acidophilus* producing lactic acid concentrations up to 6 g/L with final cell concentrations of 9.5 $\log_{10}$ CFU/mL (Camacho *et al.* 1991). In maize porridge, probiotic strains of *L. acidophilus* and *L. reuteri* also achieved efficacious levels of viability of 7.4 and 7.7 $\log_{10}$ CFU/mL (Helland *et al.* 2003).

The growth of *L. acidophilus* (NCIMB 8821), *L. reuteri* (NCIMB 11951) and *L. plantarum* in our experiments can be compared with other tests performed in similar cereal substrates. Though previous works report on the microbial growth of probiotic strains in media produced from whole grains, the biomass production in broths obtained

241 from debranning fractions has not been studied. It has been found here that the medium
242 from fraction A (1-3% pearling fraction) gave a maximum biomass over 9.2 $\log_{10}$
243 CFU/mL, in all cases higher than the maximum growth observed in whole cereal
244 suspension. This is due to the fact that the fraction B mainly consists of aleurone cells
245 with readily available nutrients. The maximum biomass levels in fraction B were always
246 higher than in fraction C (3-4.5% pearlings) and fraction D (4.5-100% pearlings) with
247 higher starch levels.
248
249 **Product formation and nutrients uptakes**
250 Lactic acid is one of the main metabolites and pH drops according to the lactic acid
251 production. pH levels between 3.5 and 4.0 are reported to inhibit food-borne enteric
252 pathogens such as *Listeria* and *Escherichia coli* (Helland *et al*. 2004) and these were
253 achieved in broths B, C and D. Additionally, pH values between 3.8 and 4 are considered
254 acceptable for LAB fermented products (Martensonn *et al.* 2001).
255
256 TRS is the carbon source and final sugar concentrations smaller than 0.6 g/L (as observed
257 in 0-1% pearling fraction) are considered to be limiting for the growth of lactobacilli in
258 cereal substrates (Mercier *et al.* 1992). This was not observed in the 1-3%, 3-4.5% and
259 4.5-100% pearling fractions as the final TRS concentrations were between 2-3 g/L, which
260 means the carbon source was not a limiting factor. This is supported by the work of
261 Marklinder and Lonner (1994) who found that supplementing oat medium with glucose
262 had no effect on the growth of lactobacilli. Microorganisms are able to assimilate
263 nitrogen in both inorganic and organic forms, although the availability of amino acids is

264 critical for the growth of fastidious bacteria such as lactobacilli (Plesis *et al.* 1996;
265 Vescovo 1993). The observed FAN concentration generally decreased over the course of
266 the fermentations, though some small increases were observed in the lag phase. This
267 increase could be due to protease production and/or cell autolysis.
268
269
270 **CONCLUSIONS**
271
272 The most important technological issue in the development of a novel probiotic food is
273 the ability of the strain to ferment the substrate and thus increase the viable cell
274 concentration. This work revealed that three of the four broths used (5% w/v of oat
275 fractions obtained by debranning) were able to support the growth of lactobacilli above
276 the cell concentration required for functional use (10^6 $\log_{10}$ CFU/mL). Viable cells
277 reached the highest concentration and the maximum growth rates in the 1-3% pearling
278 fraction. However, limited growth was found in the 0-1% pearling fraction. *L.*
279 *plantarum* yields to the highest cell concentrations in all substrates, and *L. reuteri* shows
280 the lowest. Based on these results, the 1-3% pearling fraction is the most suitable for
281 fermentation and produces considerably higher probiotic levels, above the ones obtained
282 in whole oat flour.
283
284
285 **REFERENCES**
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406
407

FIGURE CAPTIONS

Figure 1. Debranning calibration curve.

Figure 2. Fermentation of *Lactobacillus plantarum* in oat fermentation media (A, B, C and D). Continuous lines represent the mathematical models used to fit experimental data represented by points: ○, pH; □, - Total Reducing Sugars; ◇, Free Amino Nitrogen; ●, Biomass; ■, Lactic acid. The error bars are the confidence intervals (α=0.05; n=2).

Figure 3. Fermentation of *Lactobacillus reuteri* in n oat fermentation media (A, B, C and D). Continuous lines represent the mathematical models used to fit experimental data represented by points: ○, pH; □, - Total Reducing Sugars; ◇, Free Amino Nitrogen; ●, Biomass; ■, Lactic acid. The error bars are the confidence intervals (α=0.05; n=2).

Figure 4. Fermentation of *Lactobacillus acidophilus* in oat fermentation media (A, B, C and D). Continuous lines represent the mathematical models used to fit experimental data represented by points: ○, pH; □, - Total Reducing Sugars; ◇, Free Amino Nitrogen; ●, Biomass; ■, Lactic acid. The error bars are the confidence intervals (α=0.05; n=2).

TABLE CAPTIONS

Table 1. Notation used with units.

Table 2. Chemical composition of oat fractions. ND, not detected.

Table 3. Parametric estimations corresponding to the kinetic models (1-3), applied to the cultures of *L. plantarum* in oat fermentation media obtained by debranning. CI: confidence intervals ($\alpha = 0.05$). *F*: F-Fisher test (df_1 = model degrees freedom and df_2 = error degrees freedom). r = correlation coefficient between observed and predicted data. *The lactic acid parameters (L_m and L_0) are in mg/L and v_{ml} is in mg L^{-1} h^{-1}.

Table 4. Parametric estimations corresponding to the kinetic models (1-3), applied to the cultures of *L. reuteri* in oat fermentation media obtained by debranning. CI: confidence intervals ($\alpha = 0.05$). *F*: F-Fisher test (df_1 = model degrees freedom and df_2 = error degrees freedom). r = correlation coefficient between observed and predicted data. *The lactic acid parameters (L_m *and* L_0) are in mg/L and v_{ml} is in mg L^{-1} h^{-1}. NS = Not Significant.

Table 5. Parametric estimations corresponding to the kinetic models (1-3), applied to the cultures of *L. acidophilus* in oat fermentation media obtained by debranning. CI: confidence intervals ($\alpha = 0.05$). *F*: F-Fisher test (df_1 = model degrees freedom and df_2 =

error degrees freedom). r = correlation coefficient between observed and predicted data. *The lactic acid parameters (L_m *and* L_0) are in mg/L and v_{ml} is in mg L^{-1} h^{-1}. NS = Not Significant.

FIGURE 1

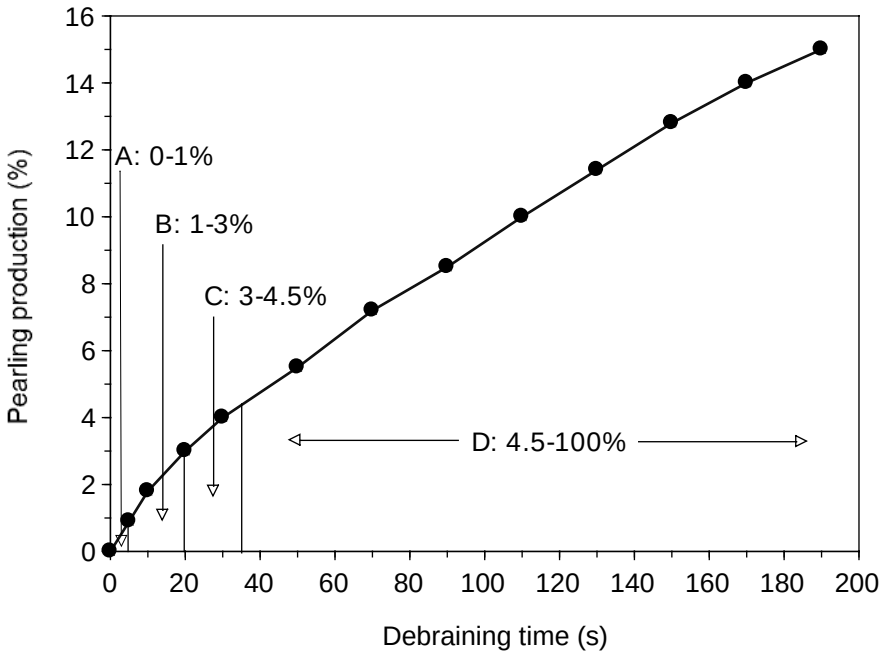
Pearling production (%)
Debraining time (s)
A: 0-1%
B: 1-3%
C: 3-4.5%
D: 4.5-100%
0
2
4
6
8
10
12
14
16
0
20
40
60
80
100
120
140
160
180
200

FIGURE 2

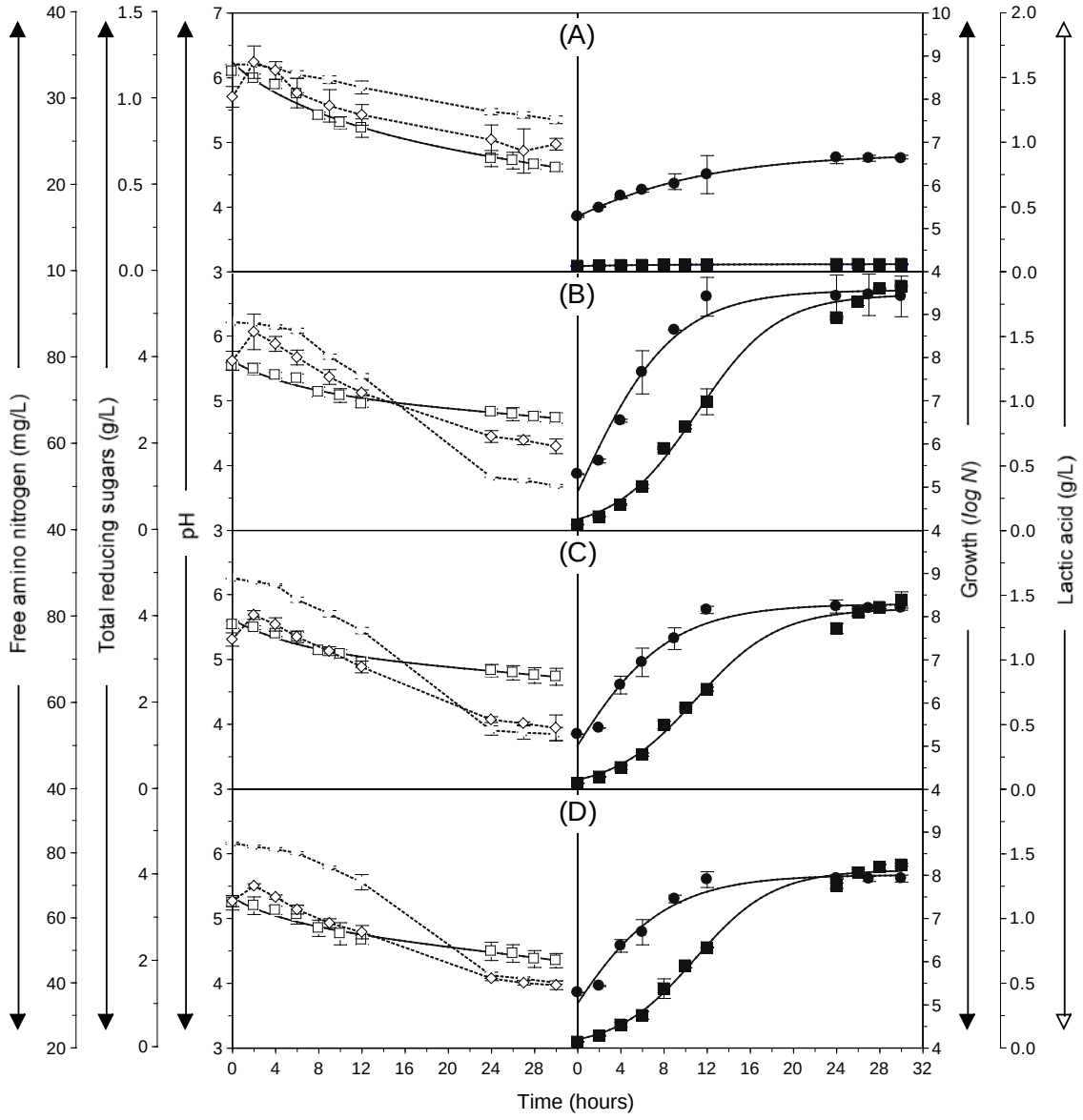
(A)
(B)
(C)
(D)
Free amino nitrogen (mg/L)
Total reducing sugars (g/L)
pH
Growth (log N)
Lactic acid (g/L)
Time (hours)

FIGURE 3

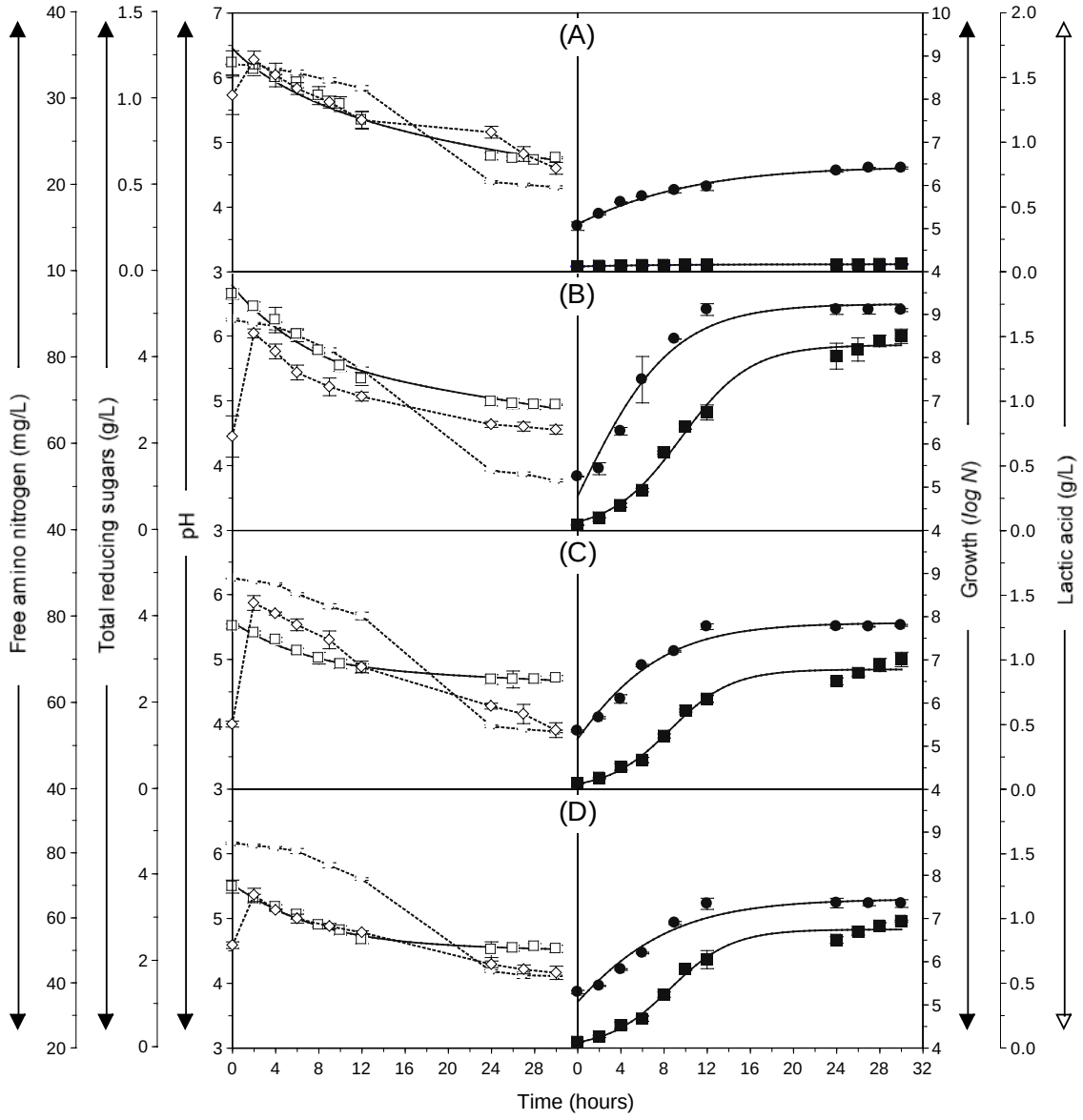
(A)
(B)
(C)
(D)
Free amino nitrogen (mg/L)
Total reducing sugars (g/L)
pH
Growth (log N)
Lactic acid (g/L)
Time (hours)

FIGURE 4

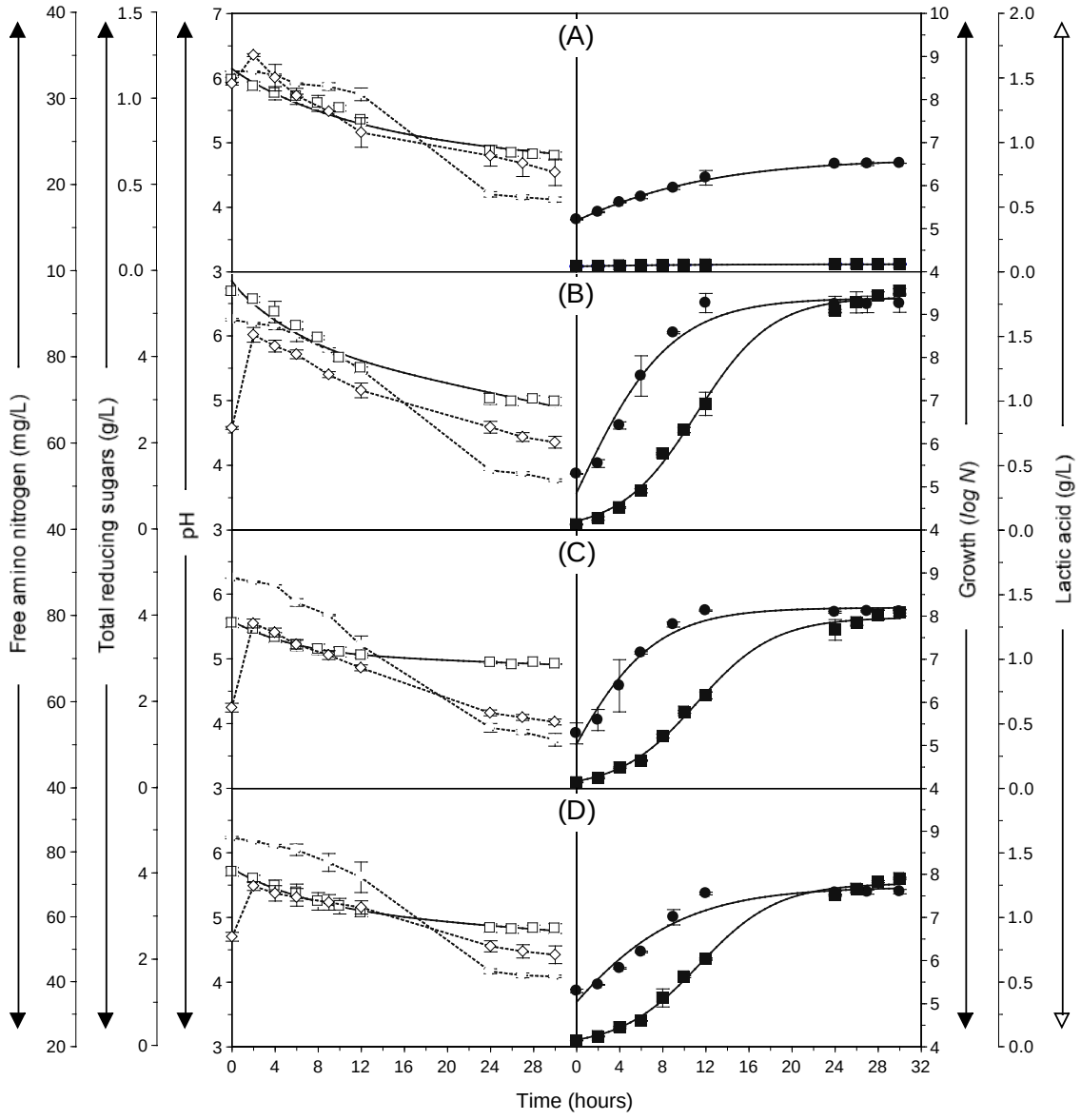
(A)
(B)
(C)
(D)
Free amino nitrogen (mg/L)
Total reducing sugars (g/L)
pH
Growth (log N)
Lactic acid (g/L)
Time (hours)

TABLE 1

X :	Biomass as logaritm of colony forming units per millilitre, $\log_{10}$ (CFU/mL)
t :	Time, h
X_m :	Maximum biomass, $\log_{10}$ (CFU/mL)
X_0 :	Initial biomass, $\log_{10}$ (CFU/mL)
v_{mx} :	Maximum growth rate, [$\log_{10}$ (CFU/mL)]/h
λ_x :	Growth lag phase, h
L :	Lactic acid concentration, g/L or mg/L (in 0-1% oat fraction)
L_m :	Maximum lactic acid, g/L or mg/L (in 0-1% oat fraction)
v_{ml} :	Maximum lactic acid production rate, g L^{-1} h^{-1} or mg L^{-1} h^{-1} (in 0-1% oat fraction)
λ_l :	Lactic acid production lag phase, h
S :	Total reducing sugars concentration, g/L
S_0 :	Initial total reducing sugars concentration, g/L
$Y_{x/s}$:	Yield coefficient for biomass formation on sugar, $\log_{10}$ (CFU/mL) g^{-1} (sugar) L
m_s :	Maintenance coefficient, g (sugar) L^{-1} [$\log_{10}$ (CFU/mL)] $^{-1}$ h^{-1}

TABLE 2

Oat Sample	Chemical Compostion (%)					
	Moisture	Protein	Total dietary Fiber	Soluble Fiber	Insoluble Fiber	β-Glucan
A: 0-1% Pearling Fraction	10.83	5.10	29.81	1.14	27.76	ND
B: 1-3% Pearling Fraction	11.24	9.09	32.34	14.56	17.46	7.43
C: 3-4.5% Pearling Fraction	12.45	10.81	7.23	2.83	4.31	2.12
D: 4.5-100% Pearling Fraction	12.72	9.38	5.34	1.82	3.37	1.82

TABLE 3

	OAT FERMENTATION MEDIA – *L. plantarum*			
VARIABLES	A: 0-1%	B: 1-3 %	C: 3-4.5 %	D: 4.5-100%
GROWTH (*X*)	values ± CI	values ± CI	values ± CI	values ± CI
X_m	6.735 ± 0.109	9.560 ± 0.472	8.292 ± 0.335	8.008 ± 0.325
X_0	5.278 ± 0.083	4.885 ± 0.650	5.009 ± 0.461	5.036 ± 0.455
v_{mx}	0.179 ± 0.036	0.535 ± 0.178	0.423 ± 0.143	0.410 ± 0.150
F (df_1=3, df_2=6; α=0.05)	62057.05	1850.14	3088.80	3085.33
P-value	<0.0001	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9974	0.9865	0.9867	0.9845
LACTIC ACID (*L*)	values* ± CI*	values ± CI	values ± CI	values ± CI
L_m	57.131 ± 0.988	1.825 ± 0.082	1.396 ± 0.077	1.375 ± 0.059
v_{ml}	2.217 ± 0.511	0.123 ± 0.025	0.092 ± 0.023	0.095 ± 0.020
λ_l	NS	3.647 ± 0.432	3.363 ± 0.321	3.601 ± 0.331
F (df_1=3, df_2=8; α=0.05)	20301.35	2156.71	928.52	1434.39
P-value	<0.0001	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9916	0.9966	0.9960	0.9977
SUGARS (*S*)	values ± CI	values ± CI	values ± CI	values ± CI
S_0	1.214 ± 0.046	5.633 ± 0.292	3.947 ± 0.154	3.534 ± 0.163
$Y_{x/s}$	5.295 ± 1.540	2.591 ± 0.686	6.252 ± 2.070	6.400 ± 2.576
m_s	0.001 ± 0.000	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001
F (df_1=3, df_2=8; α=0.05)	4280.74	2109.10	5176.60	3393.57
P-value	<0.0001	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9904	0.9869	0.9817	0.9815

TABLE 4

	OAT FERMENTATION MEDIA – *L. reuteri*			
VARIABLES	A: 0-1%	B: 1-3 %	C: 3-4.5 %	D: 4.5-100%
GROWTH (*X*)	values ± CI	values ± CI	values ± CI	values ± CI
X_m	6.447 ± 0.124	9.241 ± 0.518	7.858 ± 0.290	7.450 ± 0.348
X_0	5.103 ± 0.111	4.785 ± 0.716	5.164 ± 0.397	5.071 ± 0.434
v_{mx}	0.188 ± 0.050	0.518 ± 0.199	0.383 ± 0.137	0.314 ± 0.147
F (df_1=3, df_2=6; α=0.05)	33707.14	1442.39	3955.52	2882.66
P-value	<0.0001	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9950	0.9821	0.9856	0.9775
LACTIC ACID (*L*)	values* ± CI*	values ± CI	values ± CI	values ± CI
L_m	56.522 ± 1.829	1.435 ± 0.066	0.927 ± 0.057	0.916 ± 0.051
v_{ml}	2.159 ± 0.787	0.112 ± 0.024	0.082 ± 0.023	0.080 ± 0.020
λ_l	NS	3.208 ± 0.312	2.932 ± 0.256	2.849 ± 0.279
F (df_1=3, df_2=8; α=0.05)	5864.97	1129.69	638.66	781.11
P-value	<0.0001	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9795	0.9965	0.9929	0.9941
SUGARS (*S*)	values ± CI	values ± CI	values ± CI	values ± CI
S_0	1.295 ± 0.056	5.689 ± 0.213	3.881 ± 0.105	3.791 ± 0.104
$Y_{x/s}$	4.500 ± 4.026	3.095 ± 0.630	2.781 ± 0.568	1.763 ± 0.325
m_s	0.001 ± 0.000	0.004 ± 0.002	0.001 ± 0.000	0.001 (NS)
F (df_1=2-3, df_2=8-9; α=0.05)	3324.74	4287.40	10202.57	9062.50
P-value	<0.0001	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9813	0.9912	0.9920	0.9947

TABLE 5

	OAT FERMENTATION MEDIA – *L. acidophilus*			
VARIABLES	A: 0-1%	B: 1-3 %	C: 3-4.5 %	D: 4.5-100%
GROWTH (*X*)	values ± CI	values ± CI	values ± CI	values ± CI
X_m	6.627 ± 0.087	9.376 ± 0.507	8.195 ± 0.310	7.706 ± 0.415
X_0	5.187 ± 0.064	4.856 ± 0.704	5.021 ± 0.467	5.039 ± 0.495
v_{mx}	0.171 ± 0.027	0.529 ± 0.197	0.482 ± 0.165	0.313 ± 0.152
F (df_1=3, df_2=6; α=0.05)	102401.7	1544.60	3223.47	2238.86
P-value	<0.0001	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9984	0.9833	0.9860	0.9762
LACTIC ACID (*L*)	values* ± CI*	values ± CI	values ± CI	values ± CI
L_m	57.261 ± 1.422	1.806 ± 0.062	1.324 ± 0.048	1.265 ± 0.045
v_{ml}	1.958 ± 0.534	0.129 ± 0.022	0.092 ± 0.016	0.087 ± 0.015
λ_l	NS	4.163 ± 0.440	3.987 ± 0.504	4.075 ± 0.498
F (df_1=3, df_2=8; α=0.05)	12055.28	2156.71	1989.23	2177.56
P-value	<0.0001	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9895	0.9984	0.9982	0.9984
SUGARS (*S*)	values ± CI	values ± CI	values ± CI	values ± CI
S_0	1.178 ± 0.030	5.792 ± 0.258	3.887 ± 0.054	4.146 ± 0.085
$Y_{x/s}$	4.200 ± 3.176	4.557 ± 1.201	4.714 ± 0.602	3.396 ± 0.595
m_s	0.001 ± 0.000	0.006 ± 0.001	0.001 ± 0.000	0.002 ± 0.000
F (df_1=3, df_2=8; α=0.05)	9761.27	3167.54	45409.06	17913.35
P-value	<0.0001	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9777	0.9874	0.9960	0.9943