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# Comparison of the Growth Kinetics of Anaerobic Gut Fungi on Wheat Straw in Batch Culture

**Bettina Bonde Nielsen[1,2], Wei-Yun Zhu[1,†], Mewa S. Dhanoa[1], Anthony P.J. Trinci[2] and Michael K. Theodorou[1,*]**

[1]*Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK*
[2]*School of Biological Sciences, 1,800 Stopford Building, University of Manchester, Manchester M13 9PL, UK*

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The growth characteristics of 17 isolates of anaerobic fungi from various animal hosts were investigated relative to their ability to degrade wheat straw. The isolates consisted of 11 *Neocallimastix* isolates from Malaysia, two *Piromyces* isolates from Australia and *Neocallimastix hurleyensis*, two polycentric isolates and a *Caecomyces* isolate, all from the U.K. These fungi were grown on wheat straw in batch culture and their characteristics determined from the wheat straw apparent dry matter (DM) loss data and a mathematical description of their fermentation gas production profiles as measured using a pressure transducer. On the basis of the apparent DM loss data, isolates were divided into three groups, viz., good (Malaysian and Australian isolates), moderate (U.K. isolates) and poor (U.K. and Australian isolates) degraders of wheat straw. From their gas production profiles, the majority of the *Neocallimastix* isolates had similar lag times, specific growth rates and fermentation gas pool sizes. However, when isolates were grouped according to their geographical location, the gas pool size and final DM loss for the U.K. isolate, *N. hurleyensis*, was significantly lower than those of the Malaysian *Neocallimastix* isolates. The apparent DM loss and gas production data for all isolates were subjected to multivariance analysis and this confirmed similarity between the Malaysian isolates. The resulting hierarchical cluster tree separated the good degraders into two distinct groups consisting of the Malaysian and Australian isolates. This research confirms that the gut fungi exhibit isolate-dependent differences in their activity towards recalcitrant substrates like wheat straw. The work also demonstrates the precision of the pressure transducer and associated modelling procedures in determining the kinetics of gut fungal growth on particulate substrates, enabling distinctions to be made between apparently similar fungal isolates.



*Address Correspondence to: Prof M.K. Theodorou, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth Ceredigion SY23 3EB, UK. Tel.: +44-1970-823-070. Fax: +44-1970-828357. E-mail: mike.theodorou@bbsrc.ac.uk
†Present address: College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China.

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## Introduction

Gut anaerobic fungi have evolved as saprophytic micro-organisms in the digestive tracts of mammalian herbivores where they are considered to participate in the primary colonisation and degradation of plant biomass [1,2], and their removal from the rumen may be detrimental to rumen function [3,4]. These fungi have been isolated from animals in a range of geographically distinct locations and are typified by their ability to colonise and degrade the recalcitrant cell walls of many wild and cultivated plants, including maize stems, rice and wheat straws, Soya hulls and temperate and tropical grasses [2,5–10]. Anaerobic fungi are known to produce a wide range of extra-cellular, plant cell-wall hydrolysing enzymes including cellulases, hemicellulases and esterases and thus they are very effective degraders of lignocellulosic materials [11–14]. For example, two isolates (*Neocallimastix hurleyensis* and an *Orpinomyces*) were able to degrade up to 60% of the apparent cell-wall biomass of wheat straw when grown in continuous flow cultures containing 80–100 g/L wheat straw dry matter (DM) [15,16].

The gut fungi have a mixed-acid fermentation converting hexose to formate, acetate, lactate and ethanol [13,17,18]. In addition to these soluble fermentation products, they produce copious quantities of gaseous end products ($CO_2$ and $H_2$) during growth. Gut fungi are usually cultured on bicarbonate buffered, habitat-simulating media. Thus, in addition to the production of $CO_2$ as a fermentation end products, $CO_2$ gas is also released from the medium (titration gas) as a consequence of the production of acidic fermentation end products (formic, acetic and lactic acids). Theodorou *et al.* [19] used a technique involving a pressure transducer to show that the gas produced by an anaerobic fungus was related to its biomass production. By using this technique with gut fungi in batch cultures, for cultures growing on soluble as well as particulate substrates, it was possible to determine the entire growth curve of the fungus in a non-destructive way. The kinetics of fungal growth could also be determined by describing the gas profiles using the gas production model of France *et al.* [20].

The production of a wide array of hydrolytic enzymes by the gut fungi, coupled with their ability to degrade recalcitrant materials, is evidence of their potential for exploitation [2]. However, only a few studies have investigated the comparative ability of gut fungi to degrade particulate substrates [11,12]; these studies were generally concerned with end point determinations of digestibility and not with the kinetics of plant cell-wall degradation. The aim of this work was to compare different gut fungi relative to their ability to degrade wheat straw by analysing growth parameters obtained by fitting pressure transducer determined gas production profiles to the mathematical model of France *et al.* [20]. In addition, the research was intended to establish if the ability of the gut fungi to degrade plant cell walls varied greatly or was similar for all isolates. The isolates studied were therefore obtained from a variety of animals (Hereford and zebu cattle, water buffalo, sheep, goats, and a grey kangaroo) and from different geographic locations (Australia, Malaysia, U.K.). Most were of the genus *Neocallimastix*, reputedly the best fibre degraders, although two *Piromyces* spp., a *Caecomyces* sp. and two polycentric isolates were also studied. These isolates were selected to provide morphological as well as geographical diversity. The phylogeny of many of the isolates used in the current study was investigated in a companion study [21]. Results from the batch culture studies with these isolates are presented and discussed herein in relation to the similarities and differences in the phenotypic expression of diversity.

## Materials and Methods

### *Micro-organisms and culture*

The following isolates were studied: (a) four from the U.K., namely *N. hurleyensis* [22] and a *Caecomyces* sp., SR4 (both from sheep) and the polycentric fungi N1 and N4 (from cattle); (b) 11 Malaysian, *Neocallimastix* isolates, from Zebu cattle (M1–M5), water buffalo (M6–M9) and goat (M10 and M11) [23]; (c) two Australian, *Piromyces* isolates, A1 from the stomach of a grey kangaroo (Theodorou, pers. comm.) and A2 from a Hereford cow [23]. Gut fungi are classified to genus level according to their thallus morphology (monocentric, polycentric) and the number of flagella associated with their zoospores (uniflagellate, polyflagallate). As the polycentric isolates N1 and N4 did not produce zoospores, it was not possible to assign them to a genus.

Gut fungal isolates from Malaysia and the Australian *Piromyces* isolate, A2, were from freshly voided faeces, the rest were obtained from digesta contents. The Malaysian and Australian fungi were obtained from animals fed on a diet consisting of grasses and other plant materials grown locally, whereas their U.K. counterparts were fed a mixture of concentrate (high in protein and low in fibre) and good quality grass as hay or silage. In comparison to the U.K. isolates, the Malaysian and Australian isolates were from animals fed on generally poorer diets with higher fibre contents.

Maintenance and experimental cultures of gut fungi were grown in batch culture at 39°C on particles of wheat straw (10 g/L, milled to pass through a 1 mm dry mesh screen) in medium C, a complex liquid medium containing rumen fluid [24]. Cultures were incubated under 100% $CO_2$ in 70 mL gas-tight serum bottles (Phase Separation Ltd, Clwyd, U.K.) sealed with butyl rubber stoppers (Bellco Glass Inc., Vineland, NJ, U.S.A.). Each bottle contained 45 mL of medium C, 0.5 g of wheat straw, 2.5 mg of chloramphenicol and 5 mL of inoculum of the appropriate isolate. The inoculum was obtained from 3-day-old cultures of the fungus grown on wheat straw in medium C as described above. Experiments were conducted using four replicates of each isolate. Four bottles of uninoculated medium C containing 0.5 g wheat straw served as the controls and were used to obtain information about the apparent DM content of the substrate following incubation.

*The pressure transducer procedure*

This technique was first described by Theodorou *et al.* [25] for nutrition research and has since been used for studying the growth of anaerobic fungi [19]. Uninoculated bottles were warmed to the incubation temperature of 39°C and their head-space gas pressure was adjusted to ambient pressure prior to and just after inoculation. Gas pressures in the head-space and corresponding gas volumes were read as described in the original publication [19,25] taking adequate asepsis precautions. The time taken for determination of pressures and volumes was not more than 10–15 s per bottle. Only a few bottles ($\leq 5$) were removed from the incubator at a time and it was assumed that the temperature (and therefore the volume) of the head-space gas remained unaltered during the measurement period. Pressure and volumes were recorded at 3–24 h intervals, readings being more frequent during active growth when head-space gas pressure increased rapidly. The fermentation was considered to be complete when less than 2 mL of gas was produced in a 24 h interval. The pH of each culture was determined at the end of the experiment and the residual biomass harvested for the determination of DM loss.

*Dry matter determination*

The weight of residual DM of the fermented wheat straw and adherent fungal biomass was determined by washing the entire culture with distilled water ($3 \times 70$ mL) through a pre-weighed filter crucibles (Sintaglass porosity 1 crucibles, 70 capacity; Gallenkamp, U.K.) using vacuum filtration. The residue was freeze-dried to constant weights and DM loss determined relative to the uninoculated control. Given that the wheat straw residue also included fungal biomass (adhering to the straw), the values obtained represent an under estimation of the true values and are referred to as apparent DM loss.

*The mathematical model*

The gas production model of France *et al.* [20] is described by the following equation:

$$Y = A - BQ^t Z^{\sqrt{t}},$$

where $Q = e^{-b}$, $Z = e^{-c}$ and $B = A\exp(bT + c\sqrt{T})$. $Y$ denotes cumulative gas production (mL), $t$ is the incubation time (h), $A$ is the asymptotic value for the gas pool size (mL), $T$ is the lag-time (h) and $b$ ($h^{-1}$) and $c$ ($h^{-0.5}$) are rate constants. In this equation, a discrete lag-time is assumed to occur before degradation commences and the specific growth rate $\underline{\mu}$ ($h^{-1}$) is time dependent and varies with time (h) as follows:

$$\mu = \frac{b + c}{2\sqrt{t}}.$$

It was decided to compare the specific growth rate at a fixed point on the growth curve. The point chosen was the time taken, after the lag phase, to reach half the final gas pool size, symbolised as $\underline{\mu}^{\frac{1}{2}}$.

*Data processing and statistics*

A PC-compatible spreadsheet program was used for data processing. This software had a linear regression facility to calculate regression-corrected values for gas volumes [19]. A Micro Vax 3600 computer (Digital Equipment Corp.) with Genstat 5 [26] and MPL [27] was used for fitting of the France *et al.* [20] model. Analyses of growth parameters and derived quantities were obtained from the fitted curves.

## Results

Cumulative gas production profiles were fitted to the model described by France *et al.* [20] and predicted values for the growth parameters were obtained. Both the experimental and predicted data showed typical growth curves containing lag time, exponential phase and stationary phase. The difference between the measured and asymptotic final gas pool size was found to be less than 5% for most isolates studied, although for the slower growing isolate, SR4, where the experimental data were not described well by the mathematical model, the difference between data sets was about 50%. This study was conducted using

**Table 1.** Growth parameters obtained from growth experiments using 17 isolates of anaerobic fungi

| Isolate | Lag time (h) | $\underline{\mu}^{1}_{2}$ ($h^{-1}$) | Predicted final gas pool (mL) | Measured final gas pool (mL) | Apparent DM loss (%) | $Y_{gas}$ (mL/g) |
|---|---|---|---|---|---|---|
| Malaysian (Zebu cattle) (*Neocallimastix* spp.) | | | | | | |
| M1 | 23.5 (2.0) | 0.031(0.0004) | 77.8 (7.8) | 77.9 (7.8) | 55.3 (1.4) | 360 (44) |
| M2 | 16.8 (1.1) | 0.025 (0.0004) | 81.4 (2.8) | 81.3 (1.0) | 58.4 (1.4) | 346(6) |
| M3 | 19.3 (1.8) | 0.029 (0.002) | 75.3 (2.6) | 75.8 (3.0) | 57.0 (0.9) | 332 (20) |
| M4 | 18.8 (1.5) | 0.024 (0.002) | 80.1 (0.7) | 79.8 (0.8) | 58.4 (0.9) | 339 (10) |
| M5 | 23.2 (3.8) | 0.026 (0.001) | 76.4 (4.7) | 73.3 (4.1) | 54.9 (1.2) | 332 (27) |
| Avg[1] | 20.3 (1.3) | 0.027 (0.001) | 78.2 (1.1) | 77.6 (1.4) | 56.4 (1.0) | 342 (5) |
| Malaysian (Water buffalo) (*Neocallimastix* spp.) | | | | | | |
| M6 | 23.9 (3.3) | 0.022 (0.004) | 78.0 (3.4) | 72.7 (2.0) | 57.3 (1.3) | 315 (9) |
| M7 | 16.0 (2.0) | 0.027 (0.002) | 82.0 (4.3) | 82.0 (4.1) | 57.5 (1.6) | 355 (19) |
| M8 | 18.5 (1.4) | 0.027 (0.0008) | 81.4 (1.8) | 81.9 (2.2) | 57.3 (0.8) | 356 (14) |
| M9 | 22.0 (4.5) | 0.023 (0.0007) | 80.3 (2.2) | 79.1 (2.3) | 53.8 (1.2) | 365 (1) |
| Avg[1] | 20.1 (1.8) | 0.025 (0.001) | 80.4 (0.9) | 78.9 (2.2) | 56.5 (0.9) | 348 (11) |
| Malaysian (Goat) (*Neocallimastix* spp.) | | | | | | |
| M10 | 15.7 (1.7) | 0.025 (0.0009) | 81.4 (1.2) | 82.2 (2.4) | 58.3 (1.1) | 352 (10) |
| M11 | 16.4 (2.4) | 0.030 (0.0007) | 77.3 (2.1) | 77.9 (2.5) | 58.0 (0.7) | 335 (16) |
| Avg[1] | 16.1 (0.4) | 0.027 (0.002) | 79.4 (2.1) | 80.1 (2.2) | 58.2 (0.2) | 344 (9) |
| Australian (Grey kangaroo) (*Piromyces* sp) | | | | | | |
| A1 | 28.0 (2.4) | 0.025 (0.002) | 90.5 (2.8) | 87.9 (4.8) | 55.6 (0.2) | 393 (23) |
| Australian (Cattle) (*Piromyces*sp) | | | | | | |
| A2 | 33.6 (0.8) | 0.022 (0.002) | 46.5 (2.6)** | 44.4 (2.4)** | 37.5 (1.0) | 296 (14) |
| U.K. (Sheep) (*Neocallimastix hurlyensis*) | | | | | | |
| R1 | 35.0 (7.4) | 0.019 (0.003) | 71.7 (3.2) | 65.9 (3.0) | 48.3 (1.2) | 339 (14) |
| U.K. (Cattle) (Polycentric spp.) | | | | | | |
| N1 | 22.1 (2.9) | 0.026 (0.001) | 70.7 (3.4) | 70.7 (2.9) | 50.3 (0.9) | 350 (21) |
| N4[2] | 17.6 (0.7) | 0.027 (0.0006) | 72.0 (4.0) | 72.3 (2.8) | 46.4 (3.5) | 362 (13) |
| Avg[1] | 19.9 (2.3) | 0.027 (0.0004) | 71.4 (0.7) | 71.5 (0.8) | 48.4 (2.0) | 356 (6) |
| (*Caecomyces* sp.) | | | | | | |
| SR4 | 40.0 (6.4) | 0.009** (0.002) | 52.9 (4.7)** | 35.2 (1.8)** | 37.6 (3.8) | 236 (16)** |
| Avg[3] | 23.0 (1.8) | 0.026 (0.0008) | 78.4 (1.3) | 77.4 (1.5) | 53.1 (1.7) | 339.0 (8) |

Values are the means of three growth experiments; the growth parameters were obtained by fitting the gas production profiles to the model described by France *et al.* [20]. DM loss of the wheat straw due to fungal degradation was determined using a gravimetric method. Numbers in parentheses represent standard error. R1, *Neocallimastix hurleyensis*; $Y_{gas}$, mL gas produced per gram wheat straw apparently degraded.

[1]Average for groups of isolates.

[2]Mean of two growth experiments

[3]Average excluded identified outliers.

** Statistical outliers ($P < 0.01$).

well-buffered medium and pH changes during fermentations were negligible, with recorded pH values being within the range 6.5–6.7. Other values, for the parameters, derived from curve fitting and the apparent DM loss measurements are shown in Table 1, where each value is the mean of three independent experiments. Correlation between corresponding values for growth parameters from fitted curves and from DM loss data was determined using linear regression. Correlations were found between: lag time (h) and $\underline{\mu}$ at half final gas pool ($h^{-1}$) ($r = 0.88$, $P < 0.001$) (Figure 1A); lag time (h) and final gas pool volume (mL) ($r = 0.81$, $P < 0.001$) (Figure 1B); $\underline{\mu}$ at half final gas pool ($h^{-1}$) and the final gas pool volume (mL) ($r = 0.75$, $P < 0.001$) (Figure 1C). Further, a correlation ($r = 0.98$, $P < 0.001$) was found between the measured and predicted final gas pool volumes (mL) (not shown) and between the measured final gas pool volume (mL) and the apparent DM loss (%) ($r = 0.95$, $P < 0.001$) (Figure 1D). By visual inspection of Figure 1, it was found that a majority of isolates behaved similarly with regard to growth rate, length of lag phase and final gas pool size. However, isolates A2 and SR4 in all cases and isolates R1, N1, N4 and A1 in some cases were found to lie outside the main groups.

When the apparent DM loss data were plotted against the final gas pool, the isolates divided into three distinct groups; the first containing A2 and SR4 (poor degraders), the second containing the U.K.

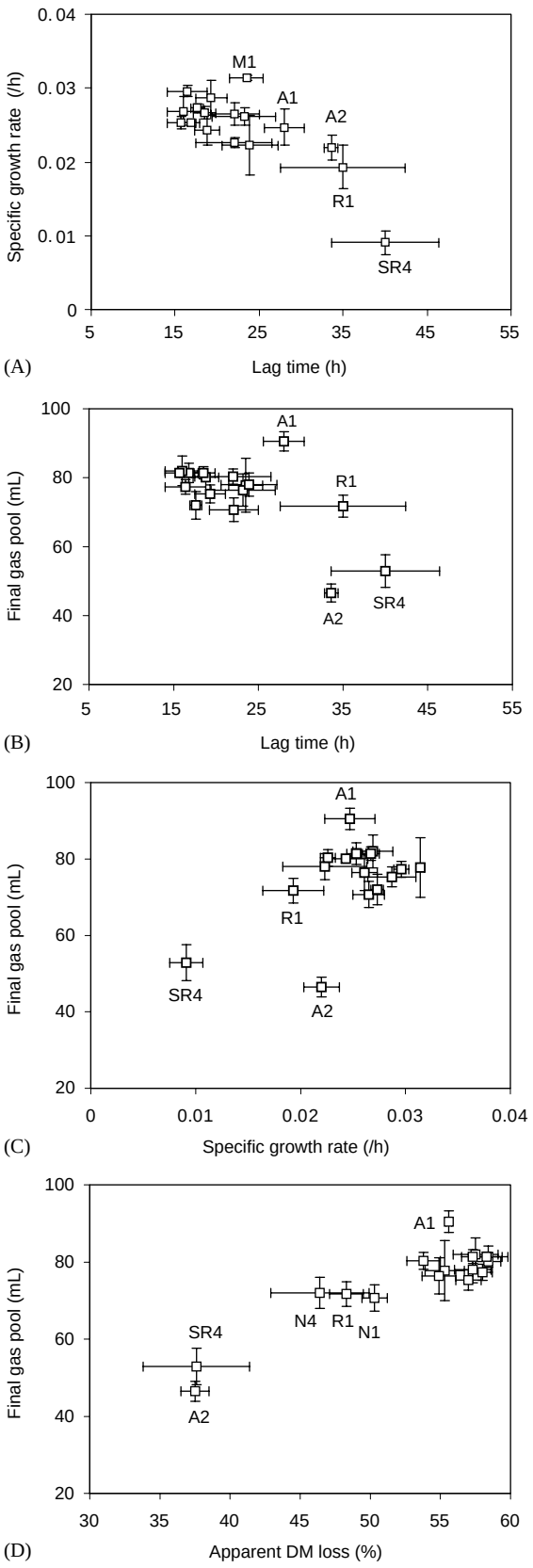


**Figure 1.** The relationship between growth parameter values of 17 isolates of anaerobic fungi. Bars represent the standard error between experiments, $n = 3$. R1 = *Neocallimastix hurleyensis*. (A) Lag time vs $\underline{\mu}$ at $\frac{1}{2}$ final gas pool. (B) Lag time vs final gas pool. (C) $\underline{\mu}$ At $\frac{1}{2}$ final gas pool vs final gas pool. (D) Apparent' DM loss vs final gas pool.

isolates, *N. hurleyensis* (R1), N1 and N4 (moderate degraders) and the third containing the Malaysian (M1–M11) and the Australian (A1) isolates (good degraders). Dixons outlier test [28] was used to analyse the values in Table 1 and both the estimated and measured final gas pool sizes produced by SR4 and A2 were found to be outliers ($P < 0.01$) as were the $\underline{\mu}^{\frac{1}{2}}$ and gas yield ($P < 0.01$) for SR4. On exclusion of the outliers, an average value for each parameter was calculated (Table 1) as follows: lag time, 23.0 (SE=1.8) (h); $\underline{\mu}^{\frac{1}{2}}$, 0.026 (SE=0.0008) ($h^{-1}$); estimated final gas pool, 78.4 (SE = 1.3) (mL); measured final gas pool, 77.4 (SE = 1.5) (mL); apparent DM loss, 53.1 (SE = 1.7) (%); gas yield, 339 (SE = 8) ($mL/g^{-1}$).

To establish if there were growth characteristic differences between fungi isolated from different host animals, the *Neocallimastix* isolates were divided into four groups according to animal species. The Malaysian isolates formed three groups: Group 1, M1–M5 isolated from Zebu cattle; Group 2, M6–M9 isolated from water buffalo and Group 3, M10 and M11 isolated from goat. The fourth group contained *N. hurleyensis* (R1) from sheep. Each of the growth parameters and the DM loss data were analysed separately using a single factor ANOVA analysis. No significant differences were found for any of the growth parameters or the DM loss data within or between the three groups of Malaysian *Neocallimastix* isolates. However, when *N. hurleyensis* (R1) was included in the analysis, significant differences were found between the four groups of isolates for the values of lag-time ($P < 0.01$; lower for R1 than for Group 3 isolates), $\underline{\mu}^{\frac{1}{2}}$ ($P < 0.02$; lower for R1 than for Groups 1 and 3 isolates), measured gas production, ($P < 0.01$; lower for R1) and DM loss ($P < 0.001$; lower for R1), but not for the values of gas yield and predicted final gas pool.

In order to analyse the relationship between the 17 isolates in more detail, the growth parameter values were subjected to a multivariance analysis (cluster analysis) [26]. For this purpose, isolates A2 and SR4 were excluded because of their significantly reduced ability to degrade wheat straw as compared to the other isolates, i.e. if A2 and SR4 were included, small differences between the main group of isolates would be 'hidden' due to the greater differences between isolates A2 or SR4 and the main group. This analysis involved calculation of the inter-group distances of isolates in relation to their growth and degradation characteristics. From this information, the relative similarity between isolates was calculated and the data were then used to create a hierarchical cluster tree that identified groups of isolates at different similarity levels (Figure 2). At approximately 95% relative similarity, the hierarchical cluster tree (Figure 2) showed two groups of isolates comprising

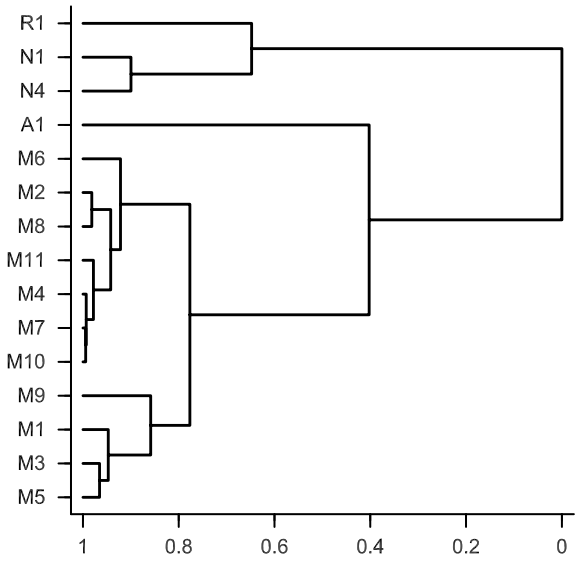


**Figure 2.** Hierarchical cluster tree of 15 isolates of anaerobic fungi based on their growth kinetics. The hierarchical cluster tree was created using multivariance analysis [26] on growth parameters and apparent DM loss data. Isolates SR4 and A2 were removed from this analysis using Dixons outlier test.

of M1, M3 and M5 in one group, and M2, M4, M7, M8, M10 and M11 in a second group; the remaining isolates were un-grouped. The isolates M6, M9, N1 and N4 were grouped however at 87% relative similarity; *N. hurleyensis* at 65% relative similarity and the isolate A1 at the 40% relative similarity level (Figure 2).

## Discussion

Fungi in the rumen are likely to be influenced by diet in much the same way as ruminal bacteria. However, although the influence of diet on bacterial populations is well established, little is known about the influence of diet on fungal populations. In this study, although it was not possible to standardise procedures to investigate the influence of diet on fungal populations, we were able to investigate potential fungal diversity by determining the growth kinetics of different fungi obtained in different ways, from different locations and different animal fed on different diets. The successful fitting of more than 50 growth curves obtained from 17 isolates of anaerobic fungi (each in triplicate) showed that the model of France *et al*. [20] was sufficiently robust to describe the growth characteristics of these organisms. This was further supported by the similarity between the measured and predicted data sets and confirmed, for example, by the highly significant correlation between the measured and predicted final gas pool sizes. Numerous other relationships were determined, for example correlations ($r > 0.75$, $P < 0.001$) between $\underline{\mu}_2^1$ ($h^{-1}$) and final gas pool (mL), lag time (h) and $\underline{\mu}_2^1$ ($h^{-1}$) and between lag time (h) and final gas pool (mL). A correlation was also found between the measured final gas pool and apparent DM loss ($r$ = 0.95, $P < 0.001$). When these parameters were plotted (Figure 1) and inspected visually, it was clear that the majority of isolates behaved in a similar way with regard to growth rate, length of lag-phase and size of the final gas pool, with R1, A1, N1 and N4 being sometimes placed and SR4 and A2 being consistently placed outside of the main group. Dixons outlier test confirmed that the measured and estimated final gas pools produced by isolates A2 and SR4 were outliers ($P < 0.01$) as was the specific growth rate $\underline{\mu}_2^1$ for SR4 ($P < 0.01$). Only isolate SR4 had a significantly lower gas yield ($P < 0.01$). It was also possible to separate the isolates into three groups namely, good degraders (Malaysian isolates and A1), moderate degraders (U.K. isolates) and poor degraders (SR4 and A2). This corresponds with the findings of Lowe *et al.* [11] and Gordon and Phillips [12], who concluded that the ability of anaerobic fungi to degrade wheat straw is dependent on genus, with *Neocallimastix* spp. and *Caecomyces* spp. representing the most and least active genera, respectively.

The *Neocallimastix* spp. were divided into groups according to source of isolation. The means of the growth kinetic parameters and apparent DM loss data were compared using a single factor ANOVAR test at 95% significant level. From this analysis, it was clear that the values for measured gas production and apparent DM loss were significantly lower for the U.K. isolate *N. hurleyensis* than for the three groups of Malaysian isolates. However, the means of $\mu_2^1$ and lag-time for *N. hurleyensis* were only significantly different to Groups 1 and 3, and Group 3, respectively. Further, there was no significant difference between the means of the growth parameters for the three Malaysian groups. This suggests that anaerobic fungi isolated from animals in Malaysia and Australia were more able to degrade plant biomass than anaerobic fungi isolated from animals in the U.K. Possible explanations include the fact that the overseas isolates were better able to cope with higher fibre diets or that they have a higher tolerance towards their fermentation end products, thus allowing fermentation to proceed to a greater extent. Previous researches proposed that some fermentation end products could inhibit anaerobic fungal growth in batch culture [29,30]. Indeed, continuous removal of fermentation products enhanced the degradation of substrate by anaerobic fungi [15,16]. On the other hand, some fungal isolates may possess more effective hydrolytic enzymes that enable them to degrade substrates more efficiently than other isolates. It is also possible that the U.K. isolates have lost some of their original ability to

degrade the plant biomass extensively. This may be particular relevant in the case of *N. hurleyensis*, for example, as this isolate has been maintained using routine laboratory subculture since its original isolation in 1983.

In conclusion, a majority (i.e. moderate and good degraders) of the anaerobic fungi studied had similar growth kinetics with similar lag-times, specific growth rates and final gas pool sizes (Figure 1) despite their origin and taxonomic status. However, in their ability to degrade wheat straw, isolates belonging to the genera *Piromyces* and *Caecomyces* were generally poorer degraders than isolates belonging to the *Neocallimastix* genus. Further, *N. hurleyensis* isolated from a U.K. sheep was found to have significantly less ability to degrade wheat straw and produced significantly less gas than the *Neocallimastix* species isolated from Malaysian Zebu cattle, Water buffalo or goat. The results also demonstrated that it is possible to use the gas production technique described by Theodorou *et al*. [19] combined with the model by France *et al*. [20] to study the growth kinetic of anaerobic fungi grown on particular substrate.

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