# $CO_2$ evolution rate during solid-state fermentation for preparation of tomato pomace as a poultry feed ingredient

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**Abstract:** $\alpha$-Tocopherol in tomato pomace fed to broilers could retard lipid oxidation in processed, heated and/or stored meat. However, in order for tomato pomace to be a value-added feed ingredient for poultry, this agricultural byproduct must contain reduced cellulose, hemicellulose and lignin, possibly achieved by amendment with Mn (487 μM/g substrate) and treatment with *Pleurotus ostreatus* under solid-state fermentation. Research was conducted to assess the $O_2$ consumption rate and the $CO_2$ evolution rate in tomato pomace treated with *Pleurotus ostreatus* without and with Mn to determine if peak colonization rate (for heightened delignification) was delayed by amendment. Results revealed that (1) one mole of $O_2$ was consumed for each mole of $CO_2$ evolved, (2) the peak $CO_2$ evolution rate for all treatments occurred between 300 to 350 h (12.5 to 14.6 d) and (3) the peak $CO_2$ evolution rate and the cumulative evolution rate were not delayed by Mn addition. Thus, when Mn was amended to tomato pomace, the metabolic activity of *P. ostreatus* was reduced, thereby overriding potential improvements in pomace delignification and *in-vitro* digestibility. An atmosphere with >20% $O_2$ and lower levels of Mn are needed to enhance delignification of tomato pomace for use in poultry feed.

**Key words:** $CO_2$ evolution, $O_2$ consumption, *Pleurotus ostreatus*, tomato pomace, poultry feed

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## 1 Introduction

Tomato pomace consists of peels, cores, culls, trimmings, seeds, liquor and unprocessed green tomatoes picked by harvest machinery. As a source of α-tocopherol in post mortem tissue, this byproduct of tomato processing could be a valuable feed ingredient in diets of poultry if it could be delignified by treatment with the edible oyster mushroom, *Pleurotus ostreatus*, before addition to feed[1,2].

Various conditions should be optimized to achieve significant bioconversion with elevated lignin degradation in an array of agricultural residues. In addition to the fungal strain, growth parameters - temperature, gaseous phase ($O_2$ and $CO_2$ concentration), particle size, substrate, and an active mediator – must be considered[3-13]. Manganese has been used as an active mediator to regulate and enhance the activity level of manganese peroxidase, improve colonization conditions and increase lignin degradation[8-13]. Giardina et al.[14] used manganese to enhance manganese peroxidase production in wood-poplar and fir culture sawdust substrates.

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To enhance delignification in a previous study on use of tomato pomace, investigators added 487 μM Mn/g substrate prior to treatment with *P. ostreatus*[15]. Mycelia of *P. ostreatus* colonized tomato pomace substrate (without Mn) under solid-state fermentation and showed degradation of cellulose and hemicellulose; however, highly indigestible lignin, a complex polysaccharide, was not degraded. Visual observation for amended (487 μM Mn/g) and treated pomace revealed a reduction in colonization by *P. ostreatus.* However, in their work on incubation conditions for colonization of pomace without and with Mn, Assi & King[15] did not measure the air supply ($O_2$) and exhaust ($CO_2$) to the fermentation. The crucial range of gaseous conditions for *P. ostreatus* delignification of tomato pomace is not known. Thus, it was not clear whether the reduction in colonization by *P. ostreatus* on tomato pomace with Mn amendment was due to $O_2$ limitations or an inhibitory effect of Mn on metabolic activity[15].

Measuring respiration rate during microbial colonization of substrates in solid-state cultivation systems has been shown to be useful for evaluating solid phase biological processes. $CO_2$ evolution rate (CER) has been used to determine the efficacy of the biolarvacide, *Lagenidium giganteum*, produced in solid-state cultivation[16]. This procedure was adapted for use in the study reported here.

Optimizing conditions for bioconversion of agricultural byproducts is crucial for the investigation of novel sources as feed ingredients in the diets of domesticated animals (a source of protein) in developed and developing countries. Thus, this study was conducted to measure the $O_2$ consumption rate (OUR) and the $CO_2$ evolution rate (CER) at one level of $O_2$ input over a 28 day period during mycelial growth and colonization of *P. ostreatus* on tomato pomace without and with Mn amendment.

## 2 Materials and methods

*Preparation of substrate*. Tomato pomace was obtained from a local processor and prepared according to methods of Assi & King[2]. Pomace was divided into two containers and $MnSO_4$ (487 μM/g substrate) was added to one container. After thoroughly mixing, substrates were placed in 250-ml Nalgene reactors, sterilized and cooled. One-half of the substrate from each container was inoculated with 5% sterile sawdust spawn[2]. Reactors (30～32℃) containing triplicate flasks of tomato pomace alone (C), C amended with Mn (CM), pomace treated with *P. ostreatus* (T) and T amended with Mn (TM) prior to colonization by *P. ostreatus* were connected to a gas flow apparatus (Figures 1 and 2) and measurements were made from repeated runs of the experiment following procedures of May & VanderGheynst[16] and VanderGheynst et al.[17]. Chemical and invitro digestibility analysis were performed on duplicate samples from two runs of the experiment for all substrates as delineated in Assi & King[15].

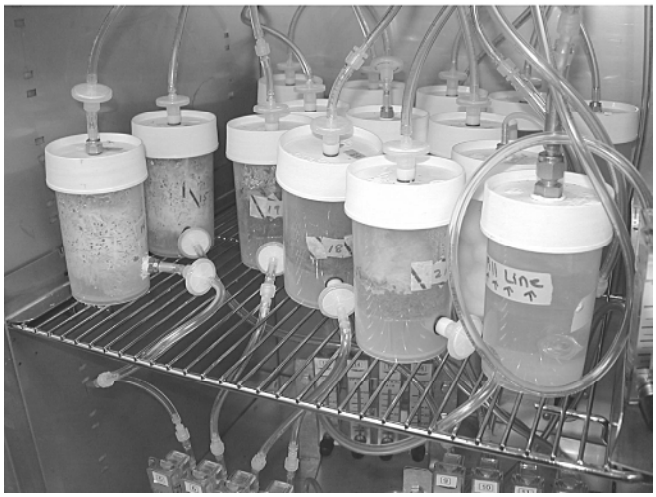

Figure 1 Incubator ( 30～32℃, air flow at 20 ml/min) with tomato pomace in reactors.

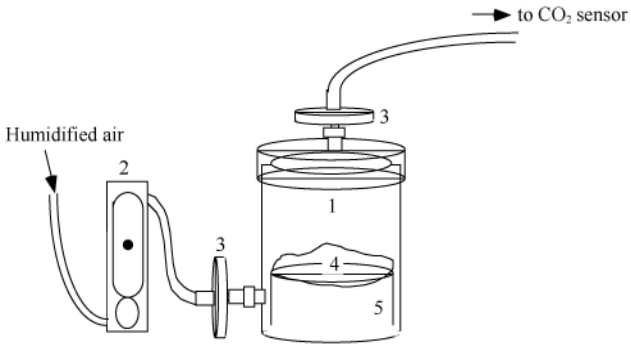


Figure 2 Schematic of reactor used to monitor pomace solid-state fermentation where 1 is 250 mL autoclavable reactor, 2 is an air flow meter, 3 is a 0.22 μm air filter, 4 is tomato pomace at 70% moisture colonized by *Pleurotus ostreatus* and 5 is support for substrate. From May and VanderGheynst (10).

*Statistical analyses.* Data were summarized in terms of peak CER and cumulative $CO_2$ evolution. These data were analyzed following a log transformation using blocked ANOVA models. For the chemistry data, variables were log-transformed to satisfy the assumption of normality and were analyzed using a 1-way ANOVA model. Unless otherwise stated, differences were considered significant at $p \leqslant 0.05$.

## 3 Results and discussion

Oxygen concentrations in the exhaust of the reactors were always ＞19% (v/v). Comparison of OUR and CER data revealed that one mole of $O_2$ was consumed for each mole of $CO_2$ evolved (Figure 3).

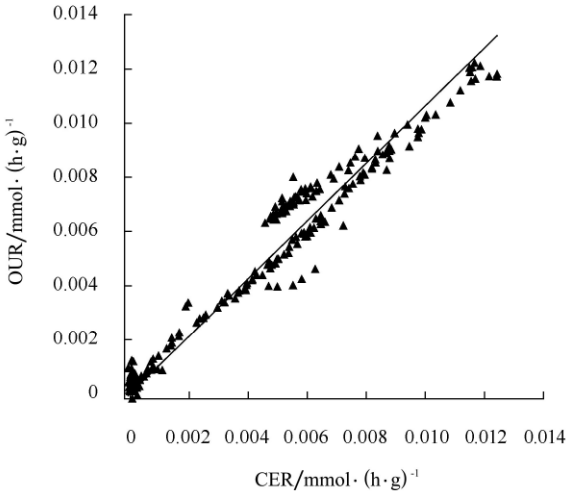


Figure 3 $O_2$ consumption rate (OUR) plotted against corresponding $CO_2$ evolution rate (CER) for *Pleurotus ostreatus* in tomato pomace. The line represents the fit of the equation y=mx. The slope of the line was 1.06 and the $R^2$ was 0.96

For this reason and because the $CO_2$ sensor had higher sensitivity compared to the $O_2$ sensor, only results for CER are shown in Figures 4～6 and Table 2.

In this study, it was assumed that full colonization occurred at the same time as peak CER. Visual observations estimated peak colonization in all substrates at about 13 days at 30～32℃. Results indicated that peak CER (Figures 4～6) for all treatments occurred between 300 and 350 h (12.5 to 14.6 days). Colonization at incubation temperatures (30～32℃) occurred five to seven days earlier than at 25±1℃(15). These results showed that in a system with a constant flow of $O_2$ at 19%, incubation occurred at 1.5X the normal rate of that in an anaerobic system. CER declined steadily after the peak period until day 19 and remained unchanged until day 28 at the end of the experiment.

Figure 4 shows that there were no other organisms present to produce appreciable fermentation as pomace without *P. ostreatus* produced CER at baseline. The increase in protein (3.16%) and the concomitant reduction of cellulose (4.93%) and hemicellulose (6.02%) in a shorter period suggested an innovative use of this technology (SSF) for biological transformation of tomato pomace as an ingredient in poultry and other animal diets (Table 1). While the 1:1 ratio of OUR to CER and minimum $O_2$ concentration of ＞19% measured for *P. ostreatus* in tomato pomace indicated aerobic metabolism, the level of oxygen supplied did not promote accelerated lignin degradation and improve *in vitro* digestibility (Table 1). However, it is possible that greater chemical changes in protein, cellulose, hemicellulose, and possibly lignin would occur more rapidly and to a greater extent than observed if higher concentrations of $O_2$ were used in fermentation of pomace by *P. ostreatus.* According to Kamra & Zadrazil[3], in general, lignin degradation increases for many fungi when there is more than 20% $O_2$ in the atmosphere and *in vitro* digestibility is highest in pure $O_2$.

That Mn had no effect is clearly shown in Figures 5 and 6. CER for TM is nearly 10X less than that for T.

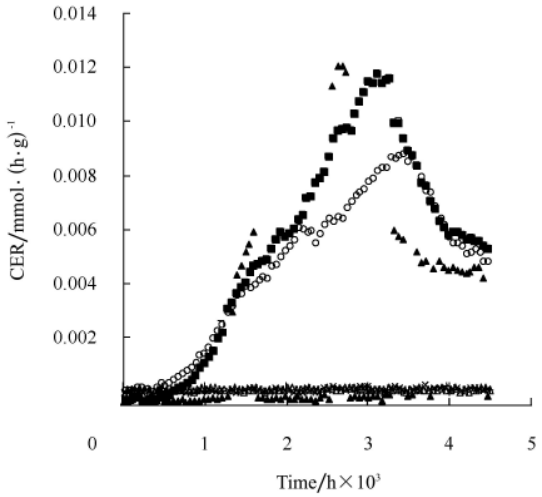


Figure 4 $CO_2$ evolution rate (CER) for tomato pomace without colonization by *Pleurotus ostreatus* (triplicate replication as x, + and Δ - all of which are at base line) and colonized by *P. ostreatus* (replications represented as ▲, ■ and ○).

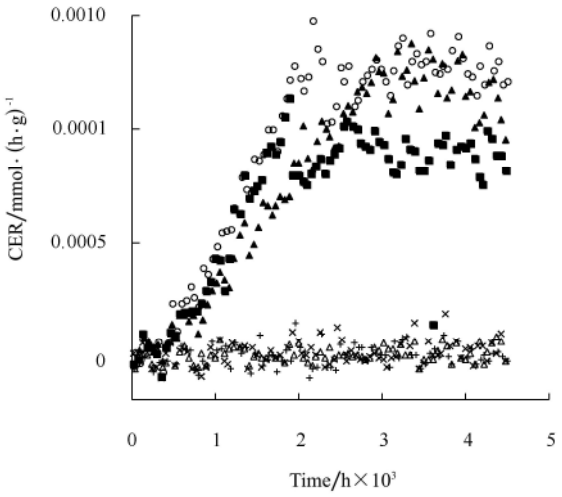


Figure 5 $CO_2$ evolution rate (CER) where ▲, ■ and ○ are triplicates of tomato pomace amended with 487 μM Mn/g substrate followed by colonization by *Pleurotus ostreatus* and x, + and Δ are triplicates of tomato pomace with Mn alone.

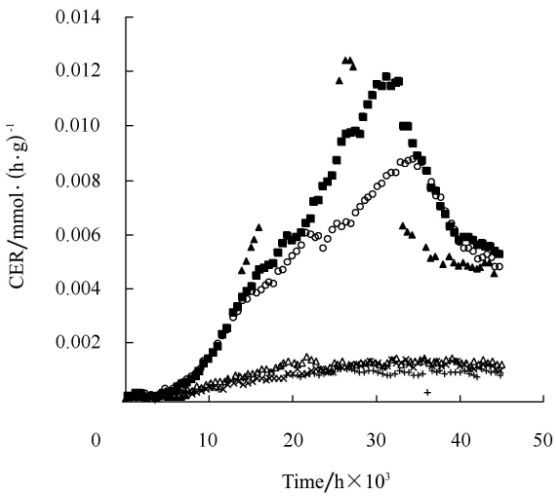


Figure 6 $CO_2$ evolution rate (CER) where ▲, ■ and ○ are triplicates of tomato pomace colonized by *Pleurotus ostreatus* and x, + and Δ are triplicates of tomato pomace amended 487μM Mn/g substrate and colonized by *Pleurotus ostreatus*..

**Table 1 Chemical composition (%) and *in vitro* digestibility (%) of tomato pomace without and with Mn amendment (487 μM/g substrate) after fermentation with *Pleurotus ostreatus* for 28 days**

| Treatments[a] | Ash | Crude Protein | ADF[b] | NDF[b] | Lignin | Cellulose | Hemi-cellulose | IVTD[b] |
|---|---|---|---|---|---|---|---|---|
| C | 3.99A | 24.22B | 47.98A | 60.53A | 29.00A | 18.98A | 12.55A | 66.83A |
| T | 4.03A | 27.38A | 45.30B | 51.83B | 31.25A | 14.05B | 6.53B | 57.00B |
| P-values(C and T) | 0.9258 | 0.0001 | O.0418 | 0.0001 | 0.1699 | 0.0399 | 0.0032 | 0.0014 |
| TM | 8.33A | 23.42B | 45.83B | 57.60A | 27.75A | 18.08B | 11.77A | 61.00B |
| P-value(T,TM) | 0.2256 | 0.0002 | 0.6598 | 0.0005 | 0.2716 | 0.3904 | 0.0094 | 0.7446 |

[a] C, control (no colonization by *P. ostreatus* and no Mn); T, tomato pomace treated with *P. ostreatus* alone and TM, T with Mn.

[b] ADF, acid detergent fiber; NDF, neutral detergent fiber and IVTD, *in vitro* digestibility; for all means (6 samples), values with different letters are significantly different at $P \leqslant 0.05$.

Table 2 shows results for total peak CER and cumulative $CO_2$. C and CM were statistically similar and different from T and TM which were similar. Theses results alone with those from Figures 4-6 revealed that $O_2$ in pomace was not delayed in time (number of days) by Mn addition. This finding was supported by observations from comparison of mushrooms produced on T and TM where those from TM appeared at the same time but were considerably smaller from those of T (Figure 7). Thus, Mn at 487 μM/g tomato pomace reduced the metabolic activity of inoculated *P. ostreatus*.

**Table 2 Log peak CER[1] and log cumulative $CO_2$ of *Pleurotus ostreatus* in tomato pomace amended without and with Mn at 487 μM/g substrate**

| Treatment[2] | Log peak CER | Log cumulative $CO_2$ |
|---|---|---|
| C | -9.63±0.26[a] | -7.02±0.77[a] |
| CM | -9.50±0.27[a] | -7.74±0.64[a] |
| T | -5.19±0.53[b] | 0.003±0.69[b] |
| TM | -6.35±0.49[b] | -1.04±0.50[b] |

[1] $CO_2$ evolution rate.

[2] C, control (no colonization by *P. ostreatus* and no Mn); CM, control with Mn alone, T, tomato pomace treated with *P. ostreatus* alone; and TM, T with Mn amendment prior to colonization with *P. ostreatus*.

[3] Mean of 6 samples, values with different letters are significantly different at $P \leqslant 0.0001$.

Figure 7 White-rot fungi, Pleurotus ostreatus, cultivated in tomato pomace substrate without and with 487 μM/g substrate $MnSO_4$ (the middle bag)

## 4 Conclusions

Accelerated incubation in a system with 19 % $O_2$ occurred at 1.5X that previously reported for anaerobic conditions. This observation suggested an innovative approach for degrading fibrous content of agricultural byproducts for use in animal feed. While $O_2$ was increased, it was not at high enough levels to degrade lignin in tomato pomace. Mn amendment (487 μM/g substrate) significantly retarded the biological activity of *P. ostreatus.*

Future studies investigating lignin degradation in tomato pomace by *P. ostreatus* should focus on increasingly higher concentrations of $O_2$ without Mn. Once the maximum level of $O_2$, causing greatest increase in protein and decrease in cellulose, hemicellulose and possibly lignin in the shortest amount of time has been reached, investigations on addition of Mn at lower levels should be conducted.

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