Effect of Different Fermentation Parameters on Lactic Acid Production from Kitchen Waste by *Lactobacillus* TY50

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Effects of different fermentation parameters including temperature, pH and oxygen on lactic acid (LA) production from kitchen waste were investigated in order to establish optimum regulating measures and increase LA yield. An open fermentation mode was employed for achieving simultaneous saccharification and fermentation of kitchen waste with *Lactobacillus* TY50 as inoculum. The results showed that 45 °C was optimum temperature for synergistic relationship between inoculated strain and indigenous strains, resulting in higher LA concentration. Continuous pH adjustment to 6.0 resulted in the similar LA concentration with intermittent pH adjustment to 7.0. However, LA productivity for continuous pH adjustment was much higher than intermittent pH adjustment. Compared to anaerobic fermentation, aerobic condition resulted in a decrease in LA concentration and an increase in acetic acid concentration. LA concentration could reach to 36.29 g L^{-1} with 1.01 g L^{-1} h^{-1} of productivity and 0.44 of yield (LA/dry mass) from kitchen waste fermented anaerobically by *Lactobacillus* TY50 at 45 °C and pH 5.5–6.0.

Key words:
Kitchen waste, lactic acid, *Lactobacillus*, fermentation

Introduction

Kitchen waste (KW) derived from households and restaurants accounts for over 40 % of the total municipal solid garbage in some metropolises of China, such as Beijing, Shanghai, Guangzhou and Tianjin, etc.[1] Due to its high water content, the incineration process is unsuitable for disposing of this kind of waste.[2] It is difficult to combust without auxiliary fuel. Incineration facilities can be damaged by temperature fluctuations, and undesirable by-products, such as dioxin-related compounds, are formed.[3] To date, the majority of KW has been treated by landfilling, which tends to result in groundwater contamination, and occupies a mass of ground.[4] KW is characterized by a high organic content and generally few compounds inhibiting bacteria.[5] As a result, it is a sound substrate for fermentative lactic acid (LA) production.[5–9]

LA is used widely in the food, pharmaceutical and chemical industries with a long history.[10] A new application of LA for polymerization to biodegradable plastics (poly-lactic acid, PLA) is attracting extensive attention.[11] PLA is a good alternative to synthetic plastics since it is biodegradable and biocompatible.[12]

In the fermentative LA production process, carbohydrates are metabolized to generate LA by lactic acid bacteria (LAB). Generally, refined sugars such as glucose and sucrose are the most commonly used substrates. In this traditional process, various nutrients in the form of e.g. yeast extract, peptone or corn steep liquor are added because LAB have limited ability to synthesize B-vitamins and amino acids.[13,14] From the cost-cutting viewpoint, it is more advantageous for LA production from organic wastes that contain sufficient carbohydrates and other nutrients for LAB proliferation.[13,15] KW can meet overall nutrient requirements of LAB. Moreover, the meal composition of Chinese people results in carbohydrates occupying a considerable proportion of KW, which is in favor of LA accumulation.

Previous studies confirmed that LA production from KW was enhanced by an open fermentation mode (non-autoclaved KW as substrate).[9,16,17] However, to the best of our knowledge, regulation and control of LA fermentation of KW has not been researched intensively. The objective of this study is to probe into the environmental factors affecting LA production from KW using a newly isolated

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Lactobacillus TY 50, in order to establish optimum regulating measures and increase LA yield.

Materials and methods

Microorganisms

Strain TY50 was used as a starter culture for LA fermentation of KW, which was isolated from KW fermented anaerobically at 50 °C. The procedures of isolation were based on the method of Wang *et al.*[17] Morphological, physiological and biochemical characteristics of TY50 are listed in Table 1, which was regarded as belonging to the genus *Lactobacillus* based on the taxonomic criteria of Kandler and Weiss[18] and Axelsson.[19] *Lactobacillus* TY50 was stored at –20 °C in Man Rogosa Sharpe (MRS)[20] broth containing φ = 20 % of glycerol and subcultured every 6 months.

Table 1 – *The phenotypic characteristics of Lactobacillus TY50*

Characteristics	*Lactobacillus* TY50
Gram stain	+
Cell shape	Short rod
Spore	–
Mobility	–
CO_2 from glucose	–
Hydrolysis of starch	–
Deamination of arginine	–
Growth at 15 °C	+
Growth at 45 °C	+
Growth at pH 4.5	+
Growth at 6.5 % NaCl	+
Growth at 8 % NaCl	–

Disposal of KW

KW, used in the following experiments, was collected from a university canteen in China. Its proximate composition is shown in Table 2. The waste was smashed by a disposer with the size of 305×250×250 mm (MGB-120, Tuda Ltd., Guangzhou), stored at –20 °C.

Glucose fermentation by Lactobacillus TY50

Anaerobic batch fermentations were conducted in duplicate in 300 mL of serum vials with 100 mL of pre-reduced medium at 40 °C. Inoculation at φ = 5 % was performed with an 18 h pre-culture.

Table 2 – *Characteristics of KW used in the experiment*

Component	Content (%)*
Dry mass	17.22
Total sugar	62.68
Starch	46.12
Crude protein	15.56
Crude lipid	18.06
Crude fiber	2.26

*The contents of all components in KW were calculated on dry mass basis.

The fermentation medium contained (per liter of distilled water): 100 g glucose, 10 g peptone, 5 g yeast extract, 2 g K_2HPO_4, 2 g triammonium citrate, 0.58 g $MgSO_4 \cdot 7H_2O$, 0.2 g $MnSO_4 \cdot H_2O$, pH 6.2. The pH was controlled at 5.5~6.0 by addition of w = 50 % of $CaCO_3$ slurry. During the process of comparing aerobic and anaerobic fermentation, the glucose concentration was decreased to 20 g L^{-1}.

Fermentation of KW for LA production

100 g of smashed KW mixed with tap water (ratio of solid to liquid was 1:12)[21] were added to a 500 mL serum vial installed with gas inflow and effluent ports without feeding other nutritional substrates. N_2 gas was input into the reactor to maintain anaerobic conditions. Inoculation at φ = 10 % of *Lactobacillus* TY50 was performed with an 18 h pre-culture. The pH was maintained at 5.5–6.0 by addition of w = 50 % of $CaCO_3$ slurry, unless otherwise stated. During the sampling, N_2 gas was sparged in the substrate. For aerobic fermentation, the open (unsealed) reactor was incubated with 100 rpm agitation speed.

Since the ratio of solid to liquid was 1:12 in KW fermentation system, the LA yield (g/g dry mass) was calculated based on the following formula:

$$Y_{LA} = C_{LA} \cdot 12/1000 \quad (1)$$

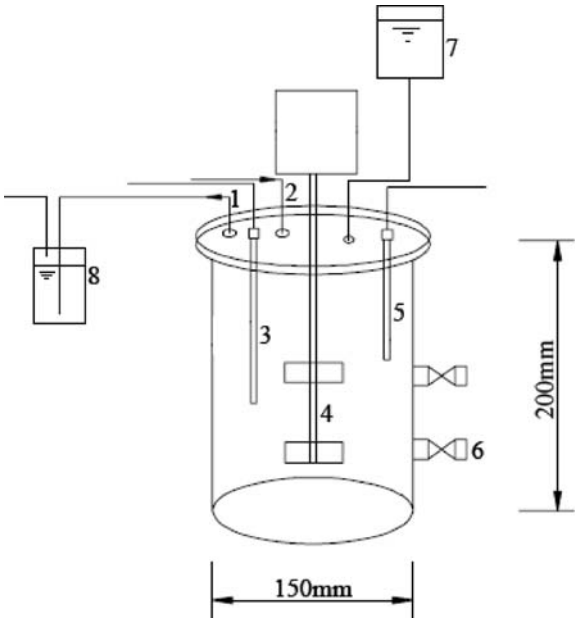
where Y_{LA} is LA yield, and C_{LA} is LA concentration (g L^{-1}).

LA productivity can be expressed as:

$$P_{LA} = C_{LA}/t \quad (2)$$

where P_{LA} is LA productivity (g L^{-1} h^{-1}), and t is fermentation time (h).

In the process of investigating the effect of pH value on LA fermentation, a cylindrical plexiglass reactor was used (Fig. 1). The reactor was completely mixed and equipped with pH control. An-



F i g . 1 – *Schematic diagram of bioreactor for LA production from KW during the study of pH effect on LA fermentation (1. gas effluent port 2. gas inlet port 3. temperature probe 4. agitator shaft 5. pH controller 6. sampling port 7. base reservior 8. gas absorption solution)*

aerobic fermentations were controlled at 45 °C, and pH was adjusted with 10 mol L^{-1} NaOH solution. Agitation speed was 100 rpm.

Analytical methods

The samples were collected at regular intervals. Each sample was filtered through 0.45 μm-pore membrane after being centrifuged at 4000 rpm for 15 minutes. The filtrate was subjected to analysis. The LA and other organic acid concentrations were measured by ion chromatography (IC) using a HPICE-AS1 column (Dionex 2010i, USA) with a conductance detector under conditions of: 1 mmol L^{-1} octanesulphur acid as eluent, TBA(OH) (tetrabutyl ammonium hydroxide) as regeneration solution, 0.9 mL min^{-1} and 25 °C.

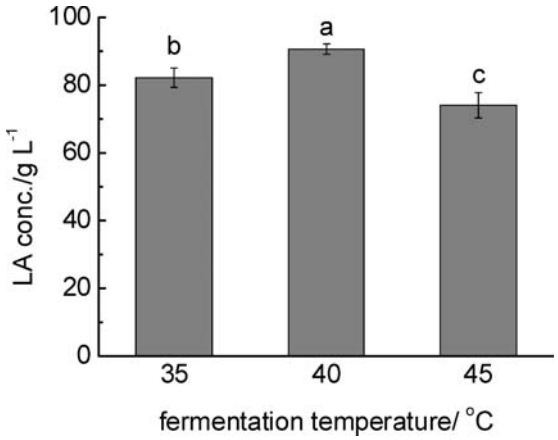
Statistical method

Duncan's multiple range test (SPSS-10) was applied using one-way analysis of variance (ANOVA). Significance is given as probability (p<0.05) values. Y-error bars and ± indicate the standard error of means among three parallel replicates.

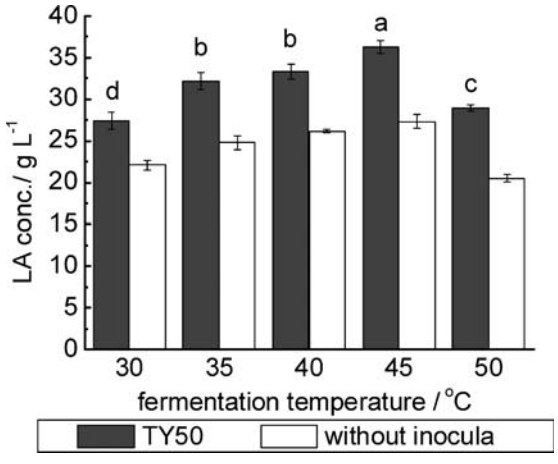
Results and discussion

Effect of temperature on LA fermentation

Temperature is a crucial parameter affecting LA fermentation. Under pure culture condition, LA concentrations from 100 g L^{-1} glucose by *Lactobacillus* TY50 at different temperature are presented in Fig. 2. It could be concluded that the optimum temperature of LA fermentation of glucose was 40 °C according to Fig. 2. Nevertheless, the optimum temperature for LA fermentation of KW was 45 °C (Fig. 3). In the open fermentation of KW, LA accumulation attributed to synergic relationship between inoculated strain (*Lactobacillus* TY50) and indigenous microorganisms.[16,17] Although 40 °C was more suitable for LA production by *Lactobacillus* TY50, collaboration of *Lactobacillus* TY50 and indigenous microorganisms could be implemented more effectively at 45 °C. In addition, from Fig. 3, it can be concluded that LA fermentation is enhanced with inoculation of *Lactobacillus* TY50 despite the open fermentation mode employed.



F i g . 2 – *Effect of temperature on LA production from glucose by Lactobacillus TY50 after 72 h fermentation (Means with different letters differ significantly (P < 0.05))*



F i g . 3 – *Effect of temperature on LA production from KW by Lactobacillus TY50 after 36 h fermentation (Means with different letters differ significantly (P < 0.05))*

Indigenous microorganisms hydrolyzed polysaccharides into soluble sugars, which were converted to LA by LAB. Starch was the major component of polysaccharides in KW (Table 2). The ma-

jority of LAB including inoculated *Lactobacillus* TY 50 cannot hydrolyze starch.[22–24] As a kind of polysaccharide, cellulose cannot be utilized by LAB. As a result, a substantial accumulation of LA from KW had to depend on both hydrolysis of polysaccharides by indigenous microorganisms and LA fermentation by LAB. Saccharification temperature is usually higher than that of LA fermentation,[25] e.g. 55 °C is optimal for amylases,[26] and 40 °C for LA fermentation of *Lactobacillus* TY 50. According to Fig. 3, it could be concluded that the optimum temperature for LA accumulation from KW was 45 °C, which represented a compromise between the optimum conditions of the hydrolysis of polysaccharides and LA fermentation by LAB.

Effects of pH and pH adjusted mode on LA fermentation of KW

The pH has a serious influence on enzyme activities and nutrient assimilations for microorganisms. Hofvendahl and Hahn-Hagerdal[27] confirmed that the yield of LA was greatly increased using LAB to ferment whole-wheat flour hydrolysate with pH maintained at 6.0 compared to fermentation without pH control. Generally, acidic conditions are not only suitable for LAB proliferation, but also conducive to hydrolysis of polysaccharides. Fig. 4 shows the effect of pH on LA concentration. The pH ranging from 5.5 to 6.0 was in favor of LA production from KW, which was consistent with LA fermentation of other substrate.[28,29]

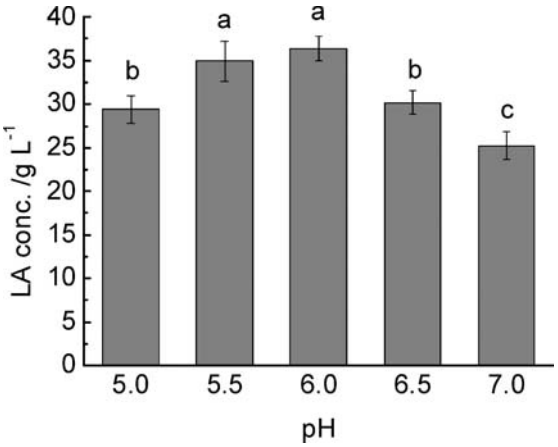


Fig. 4 – *Effect of pH on LA production from KW by Lactobacillus TY50 after 36 h fermentation (Means with different letters differ significantly (P < 0.05))*

As shown in Table 3, LA concentration in continuous pH adjustment at 6.0 was similar with intermittent pH adjustment at 7.0 with intervals of 6 h or 12 h. With intermittent pH adjustment at 7.0, the pH swings affected the metabolisms of various microorganisms in KW. For most of the fermentation

Table 3 – *Effect of pH adjustment mode on LA fermentation of KW*

Adjusted pH	Interval (h)	Concentration (g L^{-1})	Productivity* (g L^{-1} h^{-1})
6.0	0	36.38±1.42	1.01±0.04a
7.0	6	35.73±2.06	0.60±0.03b
7.0	12	35.28±1.57	0.44±0.01c

*Means with different letters differ significantly ($P < 0.05$).

period, pH was lower than 6.0 (Fig. 5), which was suitable for LAB, and unsuitable for other microorganisms. Also, in a short fermentation period, pH was close to 7.0, which was in favor of indigenous microorganisms hydrolyzing polysaccharides into soluble sugars. In succession, soluble sugars were converted to LA by LAB. Therefore, intermittent pH adjustment also resulted in a relatively high LA concentration. In contrast, the continuous pH adjustment at 6.0 led to much higher LA productivity than intermittent pH adjustment (Table 3), which was contrary to the results reported by Sakai *et al.*[16] This may be due to the different composition of KW and microorganisms used in the two systems. In our study, the fermentation period lasted about 60 h with intermittent pH adjustment to 7.0 at 6 h intervals (Fig. 5). Compared to intermittent pH adjustment, continuous pH adjustment to 6.0 underwent a shorter fermentation period (36 h), resulting in higher productivity.

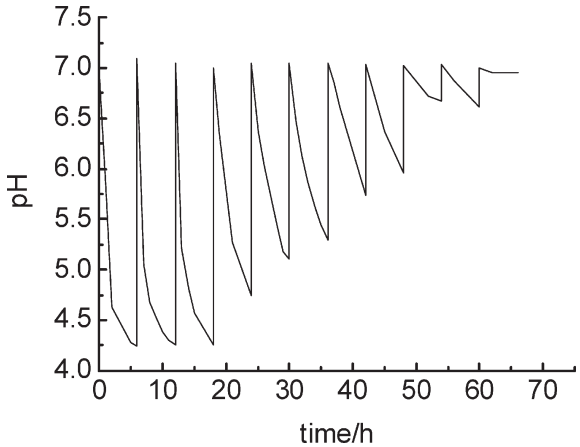


Fig. 5 – *Change in pH during the open fermentation of KW by Lactobacillus TY50 with intermittent pH adjustment at 6 h intervals*

Effect of oxygen on LA fermentation of KW

Few studies have been reported in the literature on the effect of oxygen on LA fermentation with KW as the substrate. The fermentative LA production process is usually carried out under anaerobic

conditions, since LA yield will drop with oxygen involved.[30,31] Some LAB, however, are insensitive to oxygen, and oxygen has little effect on LA yield.[26,32] According to Table 4, it can be concluded that oxygen has hardly no effect on LA fermentation of *Lactobacillus* TY 50 under pure culture conditions. With the open fermentation of KW, the effects of oxygen on LA yield and by-product production were obvious (Figs. 6 and 7). LA concentration was lower, and acetic acid concentration was higher for aerobic fermentation than for anaerobic fermentation. This suggests that the metabolic pathways of indigenous LAB in KW are transformed due to oxygen input, since oxygen does not affect LA fermentation of *Lactobacillus* TY50 as mentioned above. Oxygen can activate pyruvate dehydrogenase, or pyruvate oxidase located in some LAB, e. g. *Lactobacillus plantarum*, *L. curvatus*, and *L. sake*, etc., so that partial pyruvate is converted to acetic acid and CO_2,[30,33] which results in a decrease in LA concentration and an increase in acetic acid concentration.

Table 4 – *Anaerobic fermentation by strain TY50 on 20 g L^{-1} of glucose compared with aerobic fermentation (24 h)*

	LA (g L^{-1})	Acetic acid (g L^{-1})	Cell dry mass (g L^{-1})
Anaerobic fermentation	18.76±0.85 a	0.94±0.09 b	2.16±0.16 c
Aerobic fermentation	18.25±1.13 a	1.03±0.17 b	2.25±0.18 c

Means with the same letters within the same column differ insignificantly ($P > 0.05$).

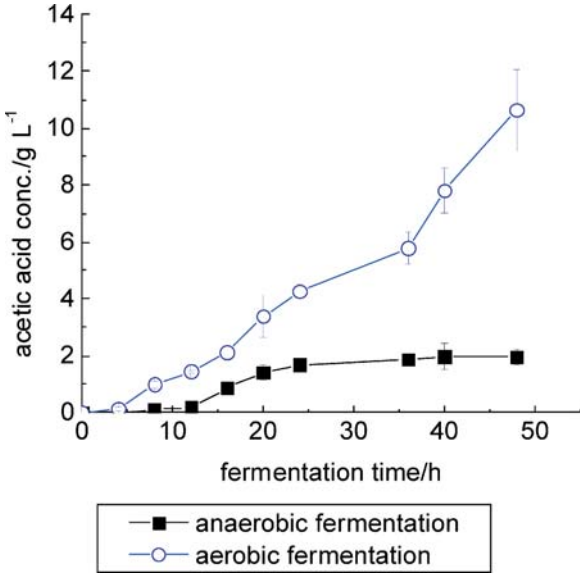


Fig. 6 – *Comparison of acetic acid concentrations between aerobic and anaerobic fermentation of KW by Lactobacillus TY50*

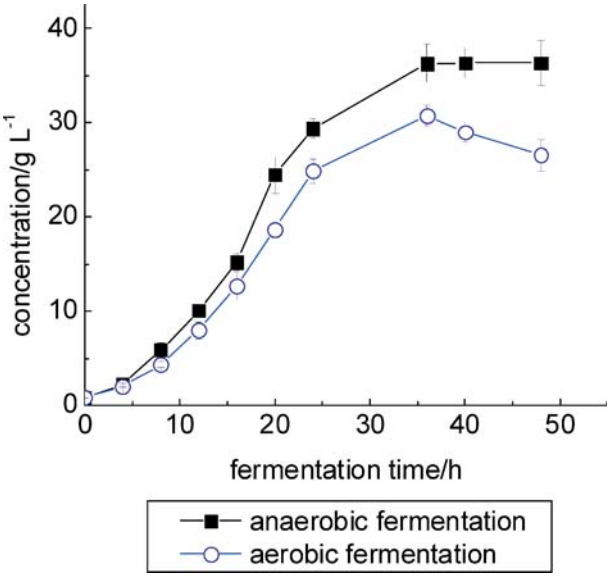


Fig. 7 – *Comparison of LA concentration between aerobic and anaerobic fermentation of KW by Lactobacillus TY50*

During the final stage of aerobic fermentation, LA concentration gradually decreased (Fig. 7), accompanied with a gradual increase in acetic acid concentration (Fig. 6). This may be because the LA was utilized as a carbon source by some indigenous LAB under aerobic conditions. For example, *L. plantarum* can use oxygen as electron acceptor, and transform LA into acetic acid accompanied by ATP generation under aerobic conditions.[30] This mechanism is advantageous for LAB, because it ensures that LAB obtain additional energy for subsistence when soluble sugars are insufficient. However, it is disadvantageous for LA production due to an increase in by-products and a decrease in LA yield.

As shown in Fig. 7, LA concentration reached 36.29 g L^{-1} with 1.01 g L^{-1} h^{-1} of productivity from KW by *Lactobacillus* TY50 after 36 h fermentation at 45 °C, pH 5.5–6.0 and anaerobic conditions. LA yield (LA/dry mass) can be calculated to be 0.44. After 36 h, LA concentration varied insignificantly with prolonged fermentation time ($P > 0.05$).

Conclusions

For the open fermentation of KW inoculated with *Lactobacillus* TY 50, environmental conditions including temperature, pH, and oxygen should be controlled in order to ensure that polysaccharides are effectively hydrolyzed by indigenous microorganisms (non-LAB), and substantial accumulation of LA by LAB.

Although the optimum fermentation temperature for *Lactobacillus* TY 50 was 40 °C, LA concentration and productivity reached maximum

value from non-autoclaved KW at 45 °C. Therefore, 45 °C was the optimum temperature for a synergistic relationship between inoculated strain and indigenous strains. The optimum pH value for LA production from KW was 5.5–6.0. Continuous pH adjustment to 6.0 resulted in similar LA concentration with intermittent pH adjustment to 7.0. However, LA productivity for continuous pH adjustment was much higher than intermittent pH adjustment due to different fermentation periods in the two systems. Compared to anaerobic fermentation, aerobic conditions resulted in a decrease in LA concentration and an increase in acetic acid concentration from KW. Since oxygen had little influence on LA production by *Lactobacillus* TY 50, the metabolic pathways of indigenous LAB in KW were transformed due to oxygen input. LA concentration could reach 36.29 g L^{-1} with 1.01 g L^{-1} h^{-1} of productivity and 0.44 yield (LA/dry mass) from KW fermented anaerobically by *Lactobacillus* TY50 at 45 °C and pH 5.5–6.0.

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List of symbols

C_{LA} – LA concentration, g L^{-1}

P_{LA} – LA productivity, g L^{-1} h^{-1}

t – fermentation time, h

w – mass fraction, %

Y_{LA} – LA yield

φ – volume fraction, %

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