Direct production of lactic acid from source-sorted organic household waste: focusing on bio-augmentation application

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Outline

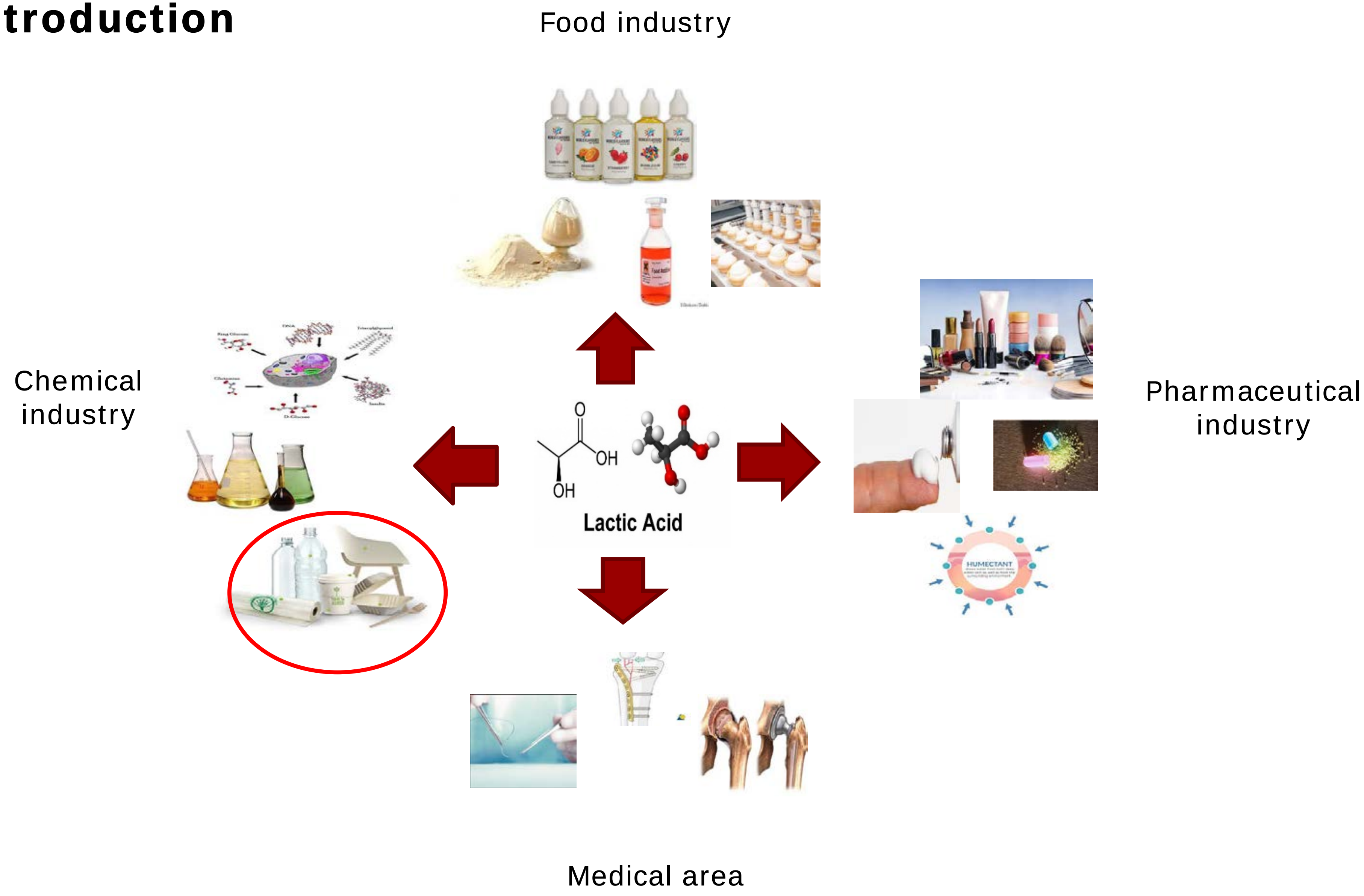
- Introduction
- Experimental design
- Result
- Conclusion

Introduction

Bioplastic

- Forty million tons of plastic will be accumulated per year in environment (Zhang et al. 2019)
- Plastics can be found in marine and terrestrial habitats, and even accumulated in the human body
- Plastic pollution creates a huge threat to the whole ecosystem

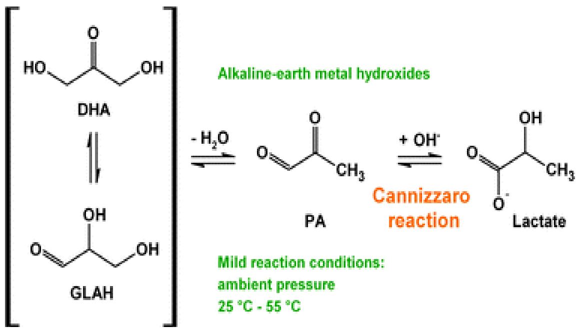
Introduction

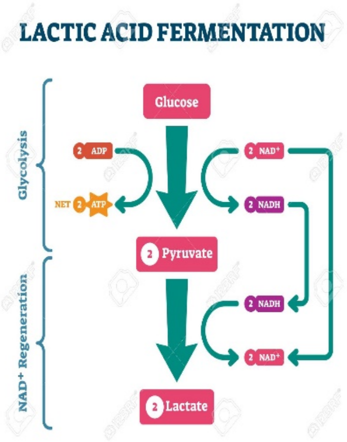


Introduction

Method for lactic acid production

Chemical synthesis





Advatages

- Rapid reaction

Disadvatges

- Mixture of L- and D- LA
- High cost
- Fossil fuels consumption

Advantages

- Accounting for above 90%
- Pure optical isomer
- Low energy input
- Environmental concerns

Challenges:

- High manufacturing cost
- Locating the suitable substrate (e.g. 90 % industry LA from corn)
- High LA yield and concentration are the eternal direction

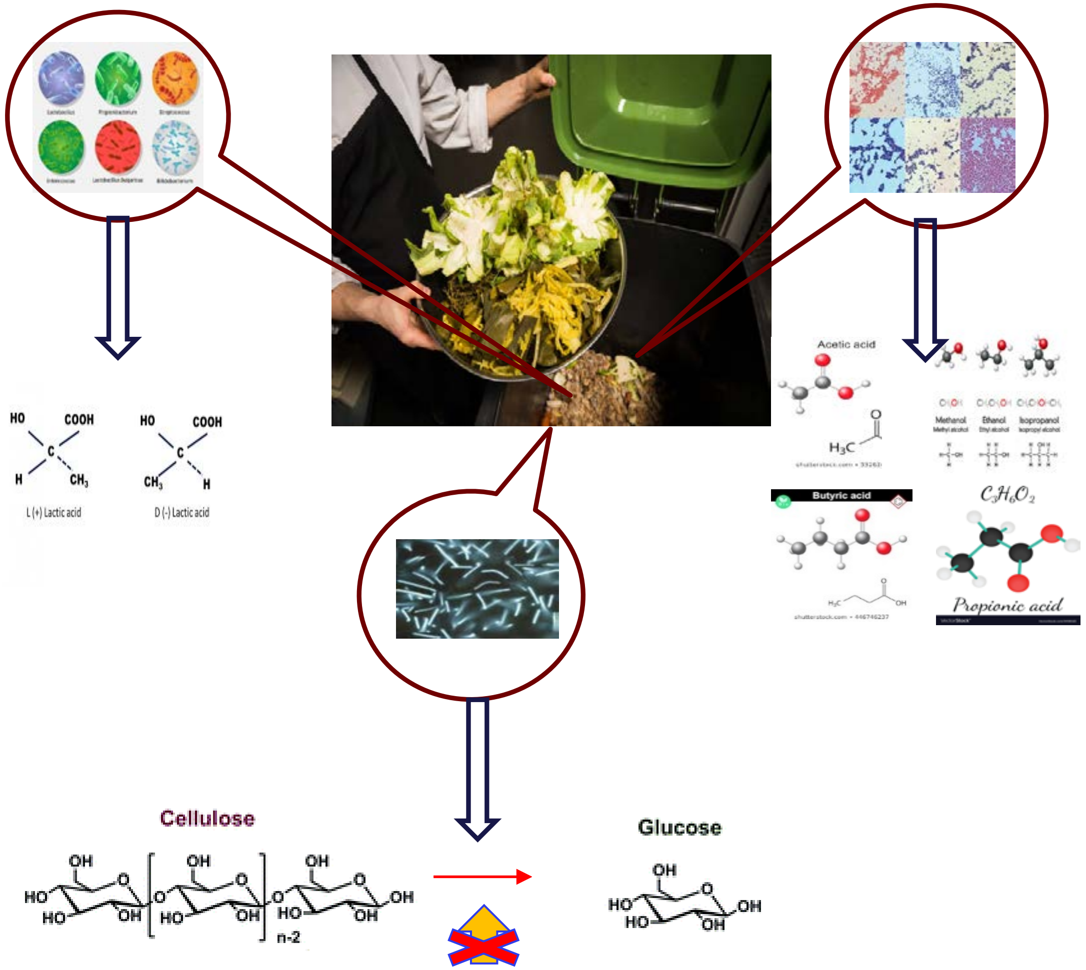
Introduction

Lactic acid production from source-sorted organic household waste (SSOHW)

- The sugar content in dry SSOHW can reach 30-40 wt%
- The sugar in SSOHW is easier to degrade than other renewable substrate (e.g. lignocellulose biomass)
- The present of hydrolytic bacteria (e.g. *Firmicutes*) degrades polysaccharose to simple sugars (e.g. glucose)
- High abundant of lactic acid bacteria in indigenous bacteria (> 85 %)

Challenges

- Acidogens (e.g. acetate and ethanol producer) competing for same substrate as the LA bacteria leading to by-product formation
- Low lactic acid yield with indigenous bacteria (< 0.5 g/g-sugar) (Tang et al. 2015)



Complex pretreatment

(e.g. High temperature, pressure and enzyme)

Aims

- **The strategy of bio-augmentation of indigenous bacteria with pure culture is used to improve LA yield from SSOHW**

- **Comparing and selecting suitable pure LA bacteria for bio-augmentation**

- **Optimization of fermentation process to further improve the LA concentration and yield**

Experimental design

Effect of bio-augmentation strategy

Control (Indigenous culture)  + Culture medium

Bio-augmentation 

Pure culture

 Sterilized SSOHW (120 °C for 20 min)

 Pasteurized SSOHW (70 °C for 30 min)

+ Inoculum

Batch experiment:

- 100 mL working volume (90 ml SSOHW + 10 mL inoculum)
- Temperature of 37 °C
- 5 days operation

Comparison of bio-augmentation strains

Control  + Culture medium

Bio-augmentation

 + Lactobacillus delbrueckii

 + Pediococcus acidilactici

Batch experiment:

- 100 mL working volume (90 ml SSOHW + 10 mL inoculum)
- Temperature of 37 °
- 5 days operation

Optimization of fermentation process

Parameters: Temperature, total solid loading and pH

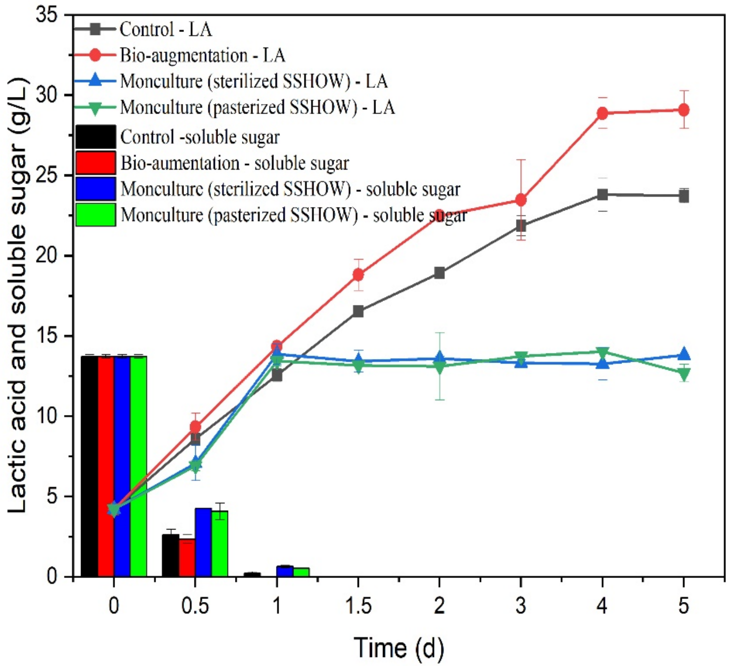
Software → Experimental design → Input: (data) → Analysis → Output → Validation

Second- order model and response surface method

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_{12}X_1X_2 + A_{13}X_1X_3 + A_{23}X_2X_3 + A_{11}X_1^2 + A_{22}X_2^2 + A_{33}X_3^2 \quad (1)$$

Result

Effect of bio-augmentation on lactic acid production



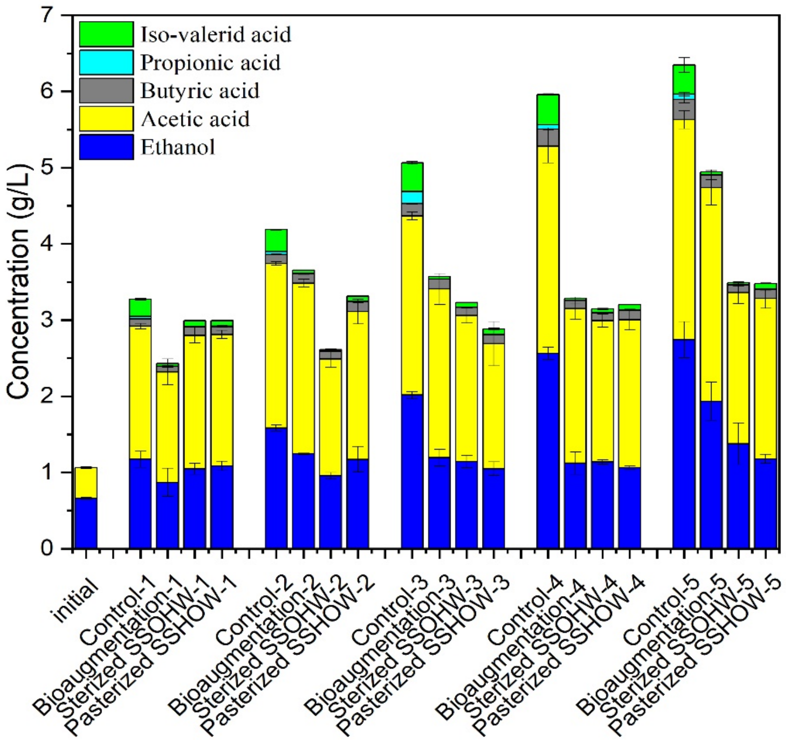


Fig. 1. Lactic acid and soluble sugar (a), and by-product concentration among monoculture culture, bio-augmentation with *P. acidilactici* and indigenous bacteria (control) fermentation.

(Soluble sugar: glucose and xylose)

- Bio-augmentation with *P. acidilactici* boosted lactic acid concentration of 109% and 22.3% compared to monoculture and indigenous culture fermentation, respectively.
- There was no significant difference between sterilized and pasteurized SSOHW
- Indigenous microorganisms played important role for decomposing complex sugars
- Bio-augmentation with *P. acidilactici* significantly decreased the by-products formation compared the indigenous culture fermentation ($p < 0.05$)

Result

Effect of bio-augmentation on lactic acid production

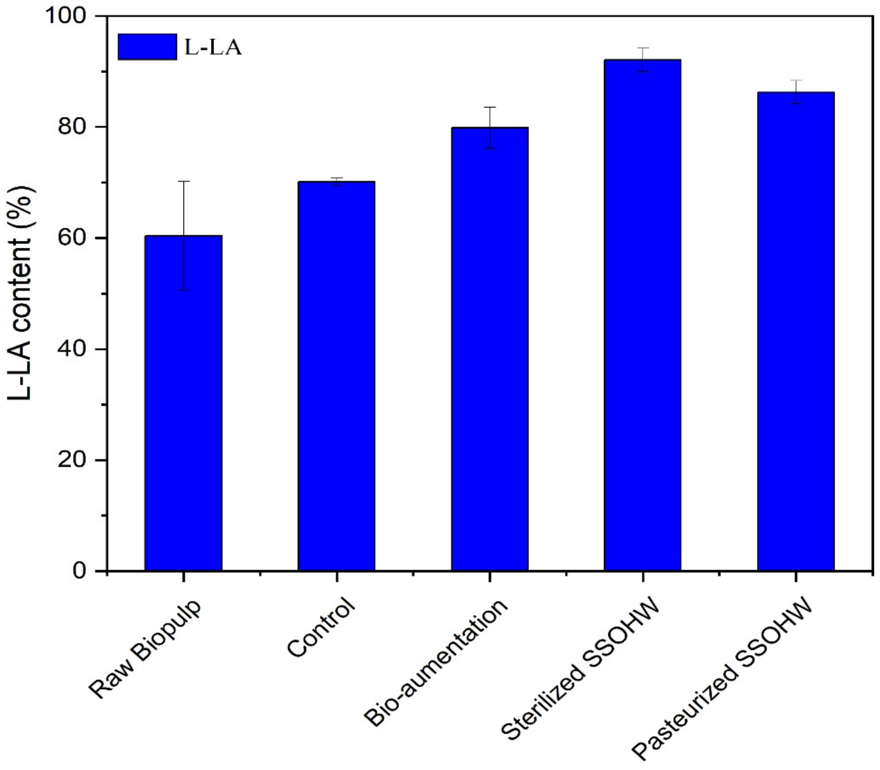


Fig. 2. The content of L-lactic acid at the end of fermentation

- The L-LA content of monoculture fermentation were 92.1% and 86.2% with sterilized and pasteurized SSHOW
- Bio-augmentation with *P. acidilactici* increased the L-LA content to 79.9% compared to indigenous culture fermentation

Result

Comparison of different bio-augmentation strains

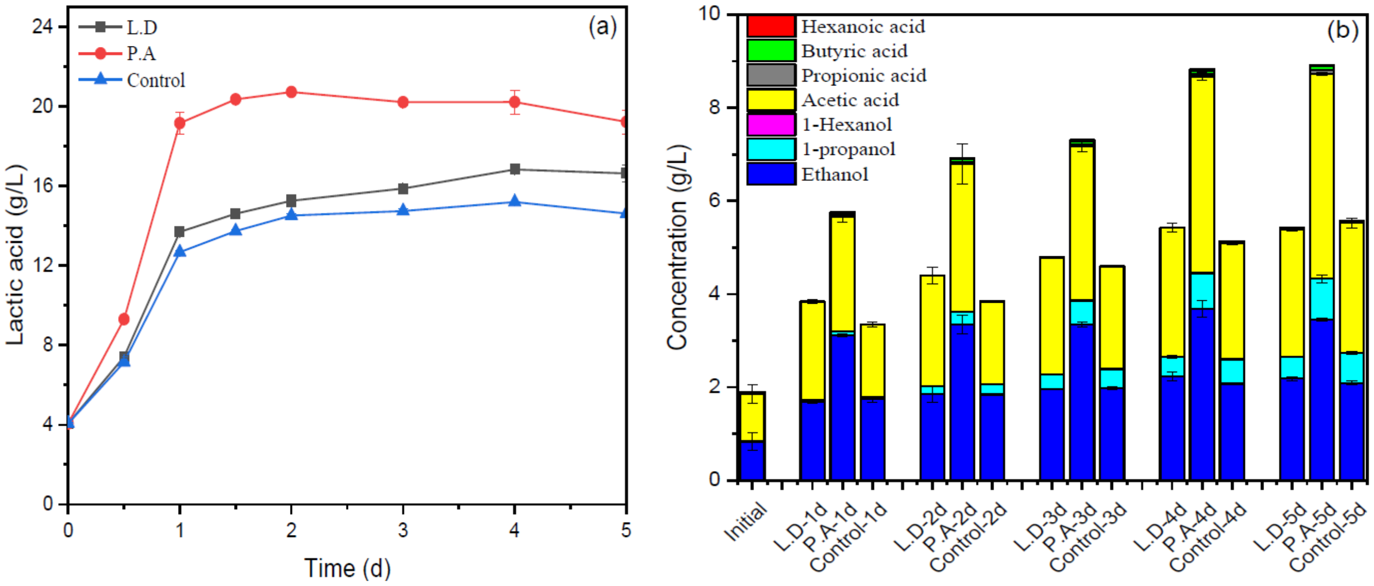


Fig. 3. Lactic acid (a) and VFAs (b) production with the addition of *Lactobacillus delbrueckii and Pediococcus acidilactici* (L.D and P.A represent *Lactobacillus delbrueckii and Pediococcus acidilactici*)

- The bio-augmentation with both strains increased the LA production
- Higher LA concentration and yield were obtained with *P. acidilactici* as inoculum compared to *L. delbrueckii*

Result

Optimization of fermentation process

Experimental design

	Temperature (°C)	pH	Total solid loading (g/L)
1	20	6.25	105
2	37	4.50	105
3	55	6.25	35
4	20	8.00	70
…			
13	37	8.00	35
14	37	6.25	70
15	37	6.25	70

Result

LA (g/L)	Predicted LA (g/L)	Yield (g_{LA}/g_{sugar})	Predicted (g_{LA}/g_{sugar})
24.20	25.34	0.57	0.57
21.40	20.89	0.50	0.51
5.60	4.46	0.40	0.39
19.40	20.25	0.68	0.71
…			
10.00	10.51	0.71	0.70
20.74	20.40	0.73	0.72
20.16	20.40	0.71	0.72

Result

Optimization of fermentation process

Table 2. Analysis of variance (ANOVA) for full quadratic model

		ANOVA				
		Sum of squares	Degree of freedom	Mean square	F-value	P-value
LA titer	Model	692.65	9	76.96	18.06	0.0067
	Lack of fit	16.95	3	5.65	62.13	0.0929
	Pure error	0.091	1	0.091		
	R^2	0.976				
	Adjusted R^2	0.922				
LA yield	Model	0.2405	9	0.0267	19.54	0.0058
	Lack of fit	0.0054	3	0.0018	15.59	0.1836
	Pure error	0.0084	1	0.0001		
	R^2	0.978				
	Adjusted R^2	0.928				

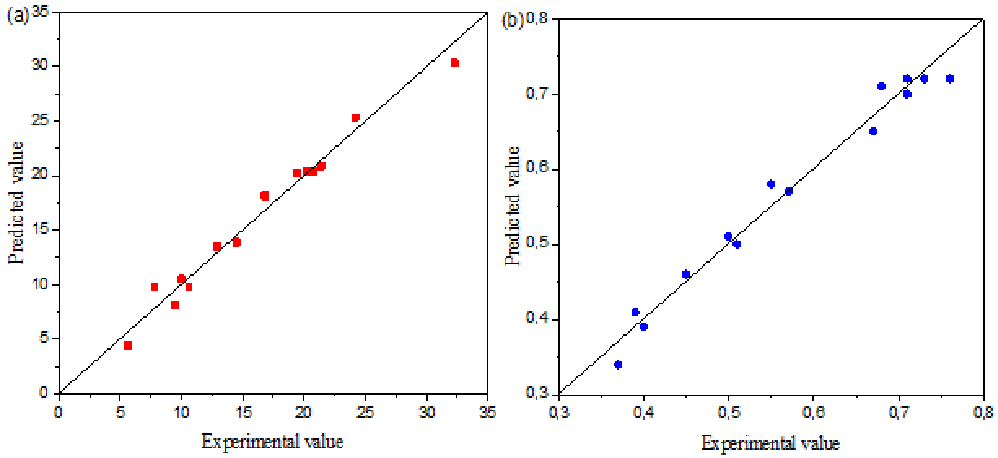


Fig. 4. The comparison of predicated value and experimental value: (a) lactic acid concentration, (b) lactic acid yield

- RSM model was significant and well-described the effect of three independent variables (T, TS and pH)
- Predicted versus experimental values clustered together, indicating model could adequately fit the experimental results

Result

Optimization of fermentation process

The experimental data was analyzed using multiple regression analysis and the second-order model equations were obtained by fitting experimental results (X_1=temperature;X_2=pH and X_3=total solid loading):

$$Y_{titer} = -33.97316 + 1.29020X_1 + 4.25510X_2 + 0.215347X_3 - 0.021224X_1X_2 - 0.001429X_1X_3 + 0.035510X_2X_3 - 0.016167\mathrm{X}_1^2 - 0.359592\,\mathrm{X}_2^2 - 0.001164\,\mathrm{X}_3^2$$

$$Y_{yield} = -0.759852 + 0.037141X_1 + 0.235645X_2 + 0.000631X_3 - 0.000748X_1X_2 + 0.000042X_1X_3 + 0.000407X_2X_3 - 0.000550\mathrm{X}_1^2 - 0.015086\,\mathrm{X}_2^2 - 0.000037\,\mathrm{X}_3^2$$

The optimum conditions for highest LA concentration and yield from software was at T=32.4, C=105 and initial pH = 8, and the predicted LA concentration and yield can reach 31.2 g/L and 0.735 g/g sugar .

Result

Optimization of fermentation process

The optimum condition of LA production from software was at T=32.4, C=120 g TS/L, initial pH = 8, and the predicted production and yield can reach 31.2 g/L and 0.735 g/g sugar.

To authenticate the output of model, duplicate Biostat A plus 3-L Fermenters (2 L working volume) were used.

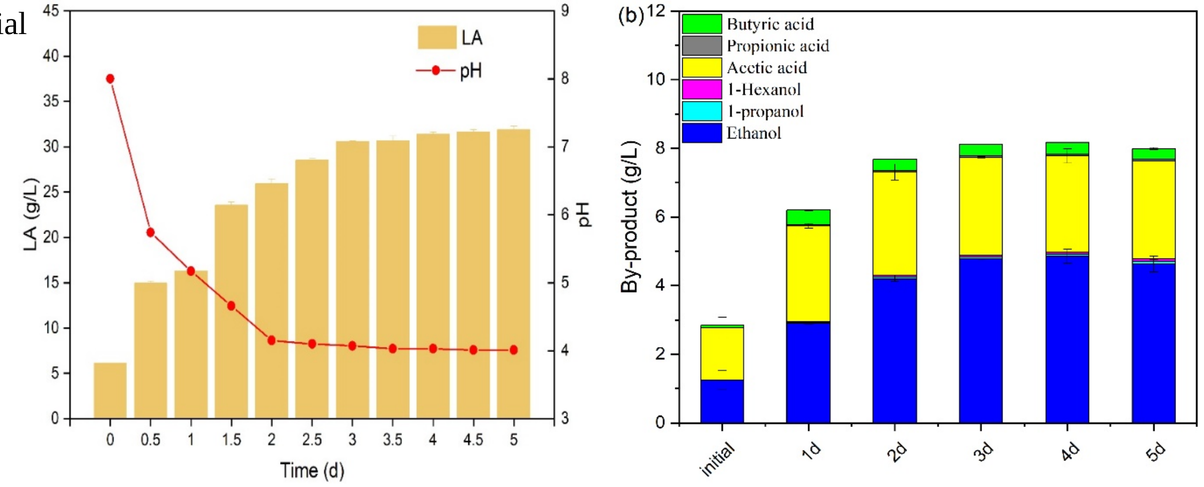


Fig. 4 Lactic acid and VFAs production of 2L fermenter at the optimum condition from model (T=32.4, C=120 g TS/L, initial pH = 8)

Initial pH: 8

Temperature: 32.4˚ C

Total solid loading: 105 g/L

The highest LA titer was 31.9 ± 0.4 g/L and the LA yield was 0.742 g/g-sugar, which was in agreement with the predicted value by the model.

Conclusion

- The strategy of bio-augmentation could significantly enhance lactic acid production and yield from SSOHW
- Indigenous microorganisms played important role for decomposing complex sugars and complex hydrolytic method (e.g. enzyme) are not needed
- *Pediococcus acidilactici* was more suitable strain for bio-augmentation compared to *Lactobacillus delbrueckii*
- The optimum temperature, initial pH and total solid loading for fermentation process were 32.4 ˚C, 8 and 105 g-TS/L, achieving lactic acid concentration of 31.9 ± 0.4 g/L and yield of 0.742 g/g-sugar.

Thanks for your attention