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Fermentation of *Phelinus baumii* MK818502 for polysaccharides and flavonoids production using defatted silkworm pupa hydrolysates as a nitrogen source

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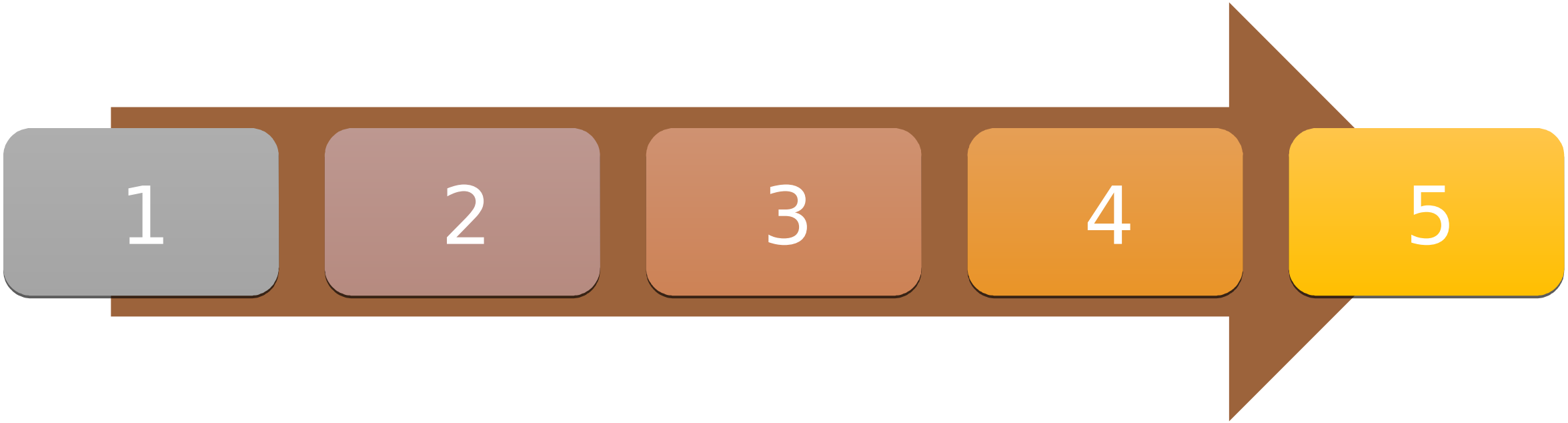
CONTENT



Introduction

Materials and Methods

Results

Conclusions

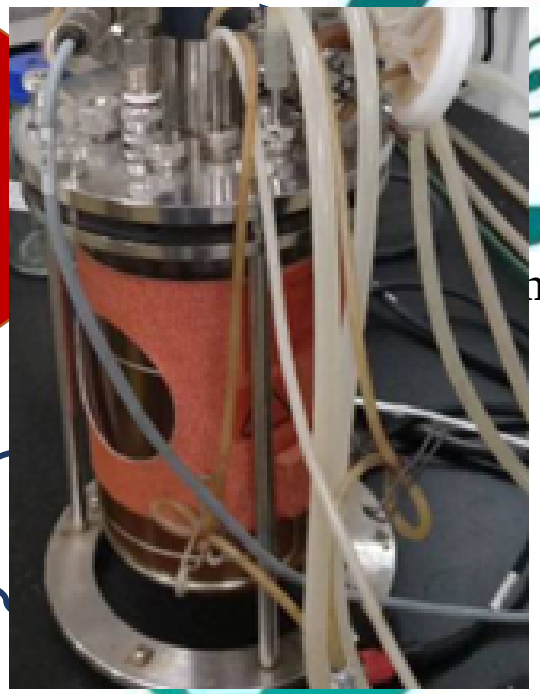
Acknowledgments

Introduction

Phelinus baumii

Polysaccharides

Flavonoids



Anti-oxidant

munity

Alternative nitrogen source ?

Anti-tumor

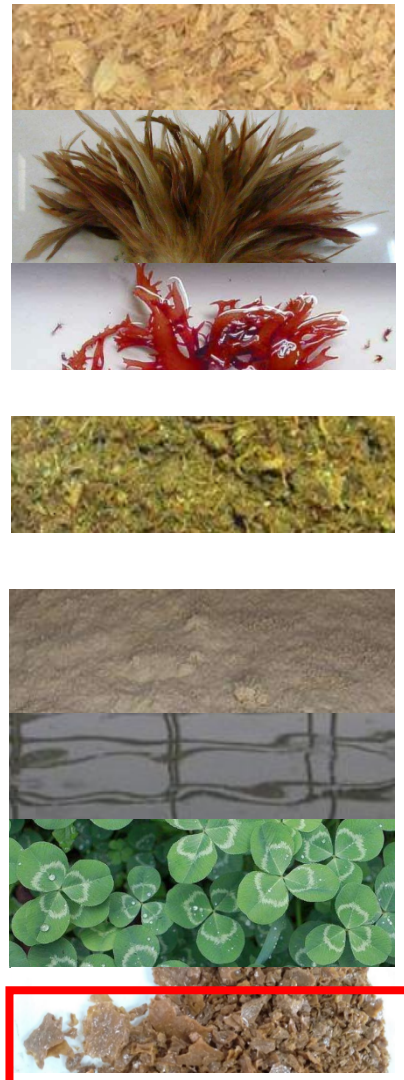
Fig. 1. A brief introduction to mulberry yellow

1. Konno, S., Chu, K., Feuer, N., Phillips, J., Choudhury, M.: Potent anticancer effects of bioactive mushroom extracts (*phellinus linteus*) on a variety of human cancer cells. Journal of Clinical Medicine Research, 7(2), 76-82 (2015)

Introduction

Table 1 The alternative nitrogen source in recent studies.

Alternative nitrogen material	Processing method	Nitrogen product	Nitrogen index (%)	Cultured organism	Reference
Rice bran	Heating	Crude protein	0.24 - 0.71	*Acetobacter xylinum*	Narh, C., etc, 2018.
Waste feathers	Hydrolysis neutralization	Chicken feather peptone	67.2	*M. purpureus* ATCC16365	Orak, T., etc, 2018.
Red seaweed	Enzymatic hydrolysis	Proteins	2 - 8.8	-	Málfríður, B etc, 2018.
Dairy manure	Anaerobically digesteddairy	Amino acid	0.25 - 0.42	*Zea mays*	Cambareri, C S., etc, 2017
Soybean residue	Solid-state fermentation	Amino nitrogen	18	*Aspergillus oryzae* TISTR 3087	Salakkam, A etc, 2017.
Fishmeal wastewater	-	γ-Polyglutamic acid	0.19 - 0.2	*Bacillus subtilis* A3	Zhang, C., etc, 2017.
White clover	Acid precipitation	Amino acids	8.35 - 20.28	*Trifolium repens* L.	Lene, S., etc, 2017.
DSPH	Enzymatic hydrolysis	Silkworm pupa protein	3.18±0.1	*P. baumii* MK818502	This study



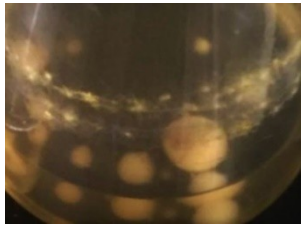
Materials and Methods

Materials and Methods

Fig. 2. Recovery and pretreatment of industrial waste silkworm chrysalis

Materials and Methods

Phelinus baumii



Shake flask culture

Single factor optimization

DSPH concentration

Temperature

pH

Revolutions per minute

Bioreactor culture

Biomass of mycelia

Polysaccharides assay

Flavones assay

Nitrogen determination

Substrate consumption

Active product generation

Dynamic fitting

Defatted silkworm pupa hydrolysates （DSPH）

Fig. 3. Technical route of DSPH as a alternative nitrogen source in fermentation of *P. baumii*

Results

Results

a

3.18 % nitrogen content

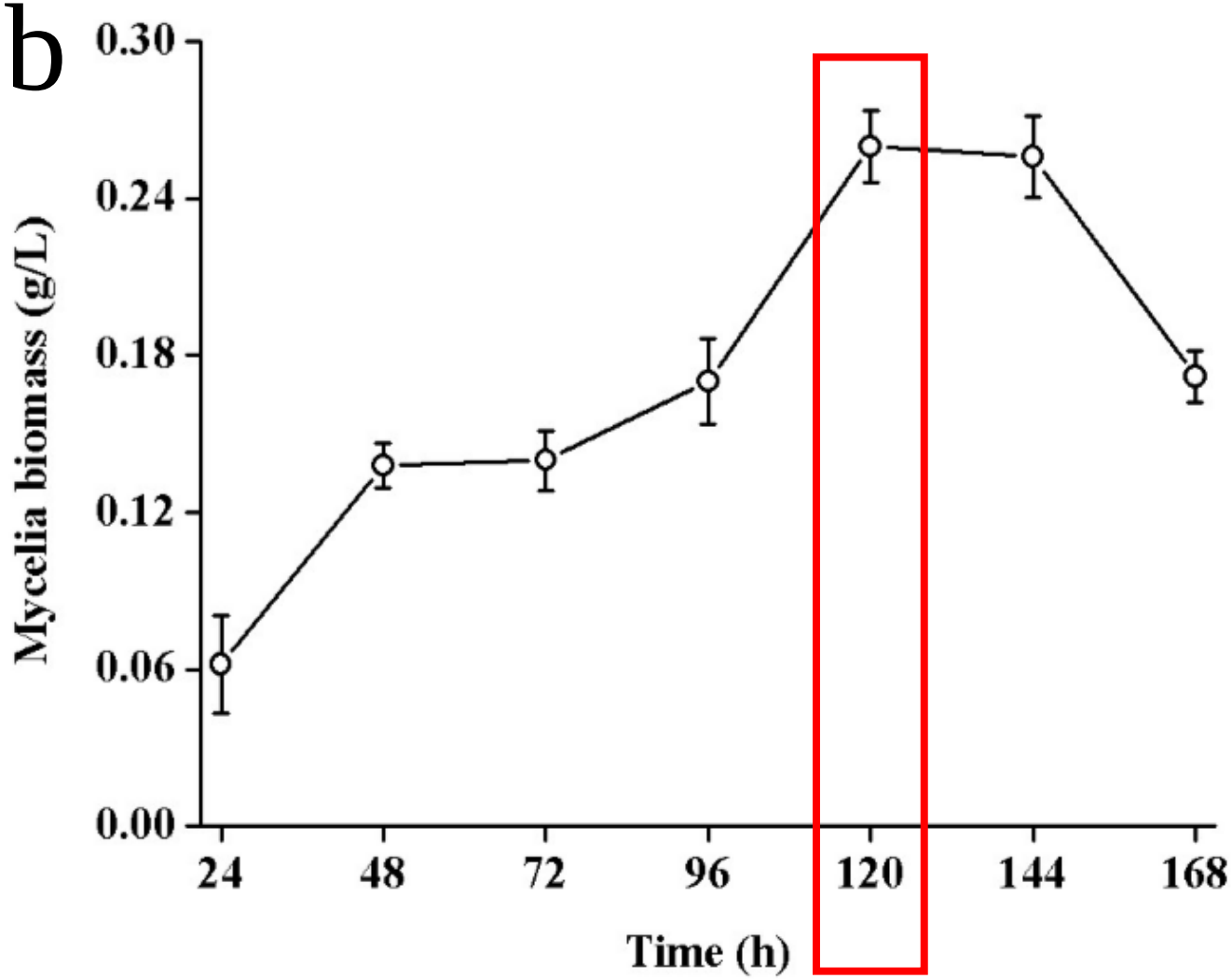
Silkworm pupae

DSPH

0.37kg

1kg

b



Extraction rate and properties of DSPH

Fig. 4. Extraction rate of DSPH (a) and the growth curve of *P. baumii* MK818502 (b) .

Results

DSPH concentration

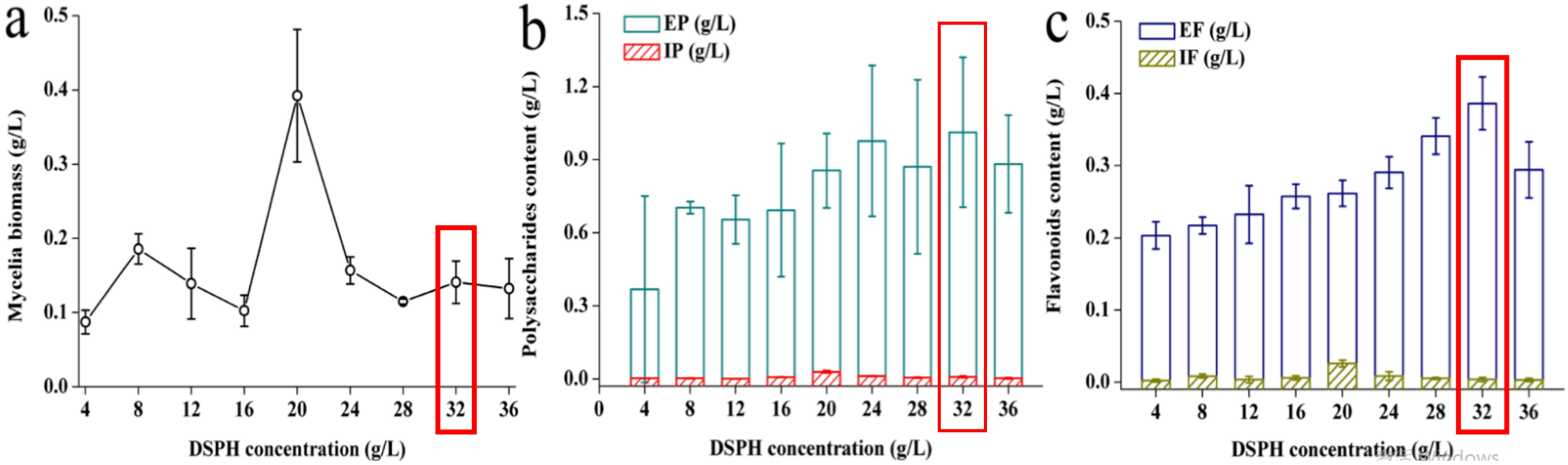


Fig. 5. Changes of related metabolites in DSPH concentration single factor experiment.

Results

Temperature

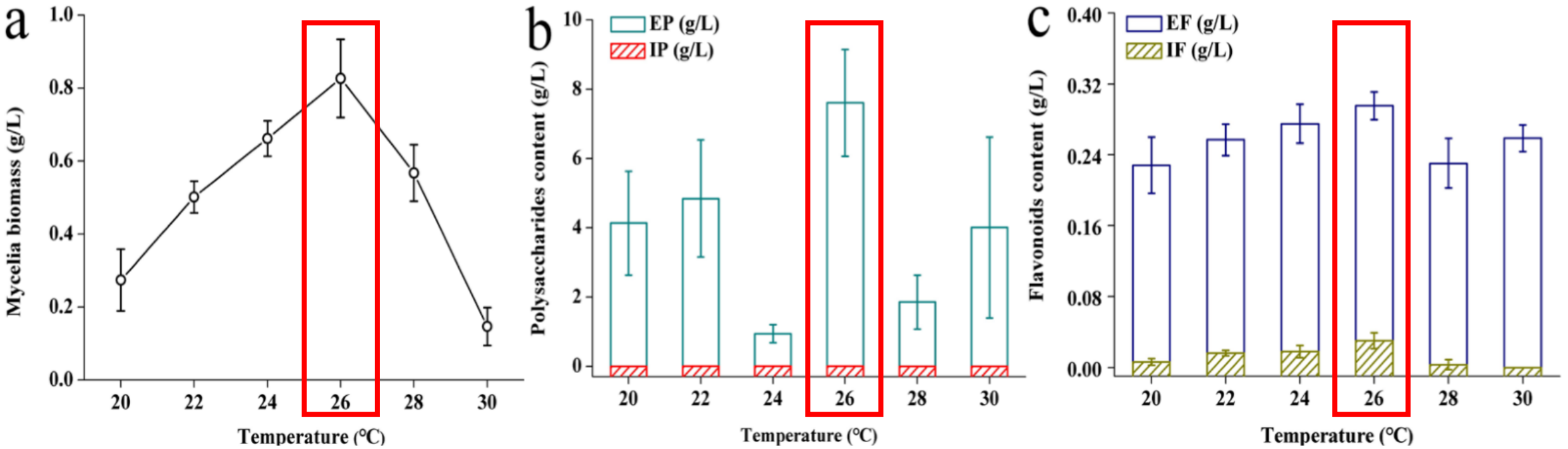


Fig. 6. Changes of related metabolites in temperature single factor experiment..

Results

pH

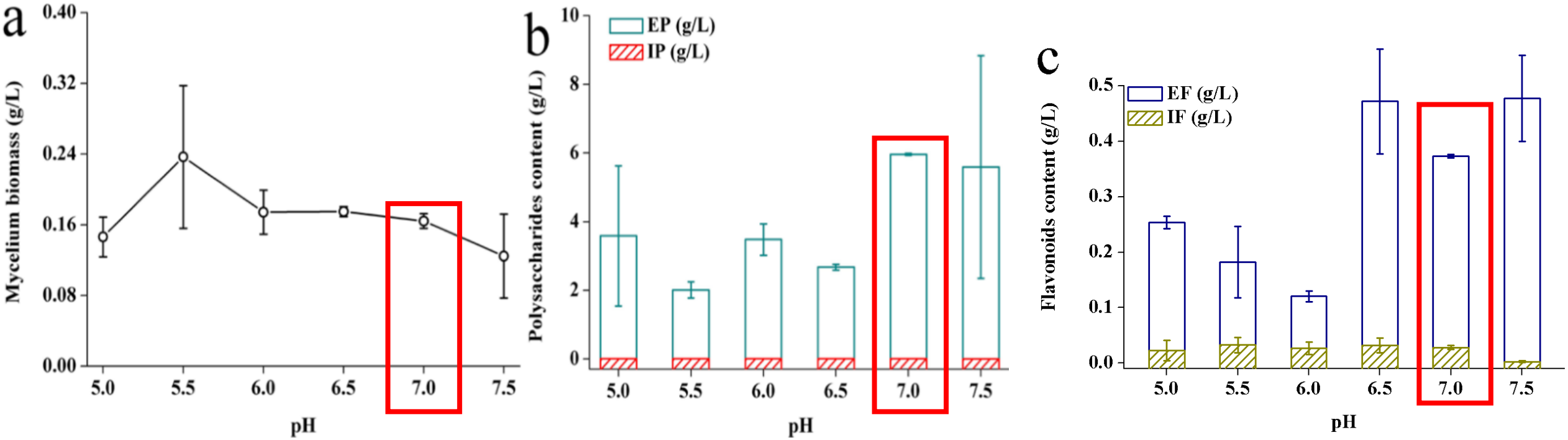


Fig. 7. Changes of related metabolites in pH single factor experiment.

Results

Revolution

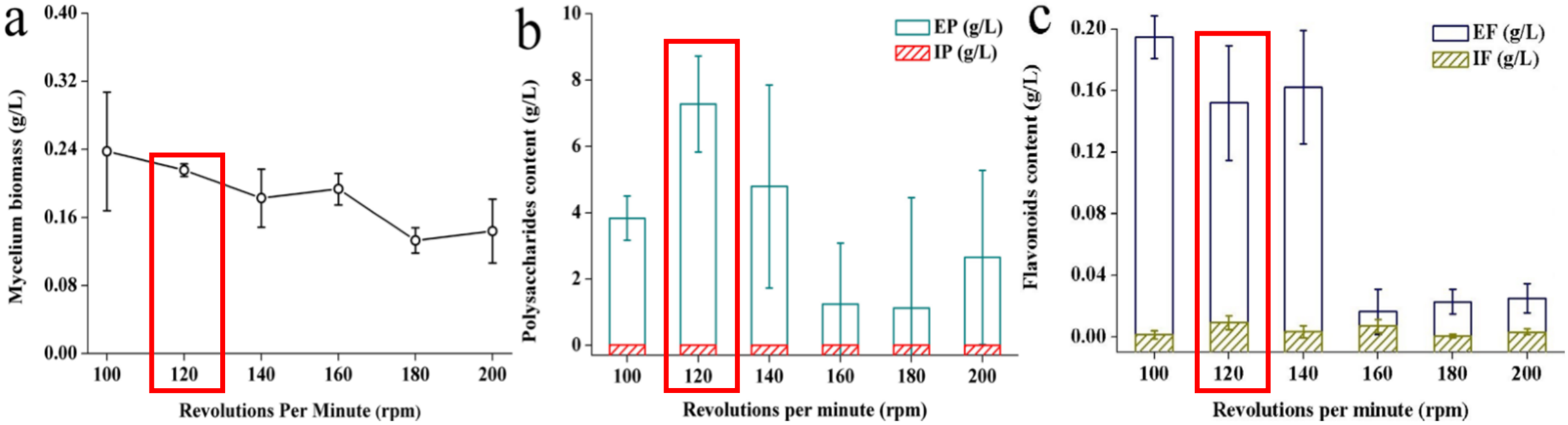


Fig. 8. Changes of related metabolites in RPM single factor experiment.

Results

Fitting formula

Biomass

$$C_x(t)=\frac{0.0132e^{0.0393t}}{0.578+0.022e^{0.0393t}}+0.0085$$

Polysaccharide

$$C_p(t)=1.1968\text{-}54.1012C_x(t)+5.5618In(0.9633+0.0367e^{0.0393t})$$

Flavonoids

$$C_f(t)=0.0643\text{-}2.3380C_x(t)+0.2397In(0.9633+0.0367e^{0.0393t})$$

Reducing sugar

$$C_s(t)=19.7868-4.8169C_x(t)-7.9359In(0.9633+0.0367e^{0.0393t})$$

Nitrogen

$$C_n(t)=0.6424-0.1707C_x(t)-0.0947In(0.9633+0.0367e^{0.393t})$$

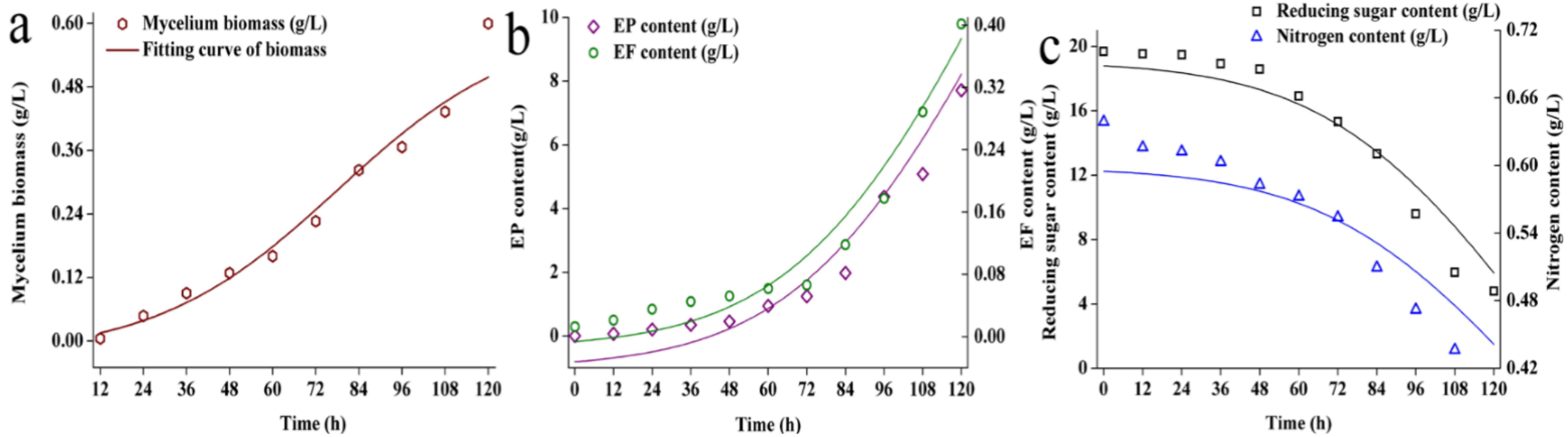


Fig. 9. Bioreactor *P.baumii* fermentation used DSPH as nitrogen source. The relevant experimental results and the fitting curve of biomass (a), extracellular product generation (b) and fermentation substrate consumption (c) were obtained..

Results

Nitrogen source contrast

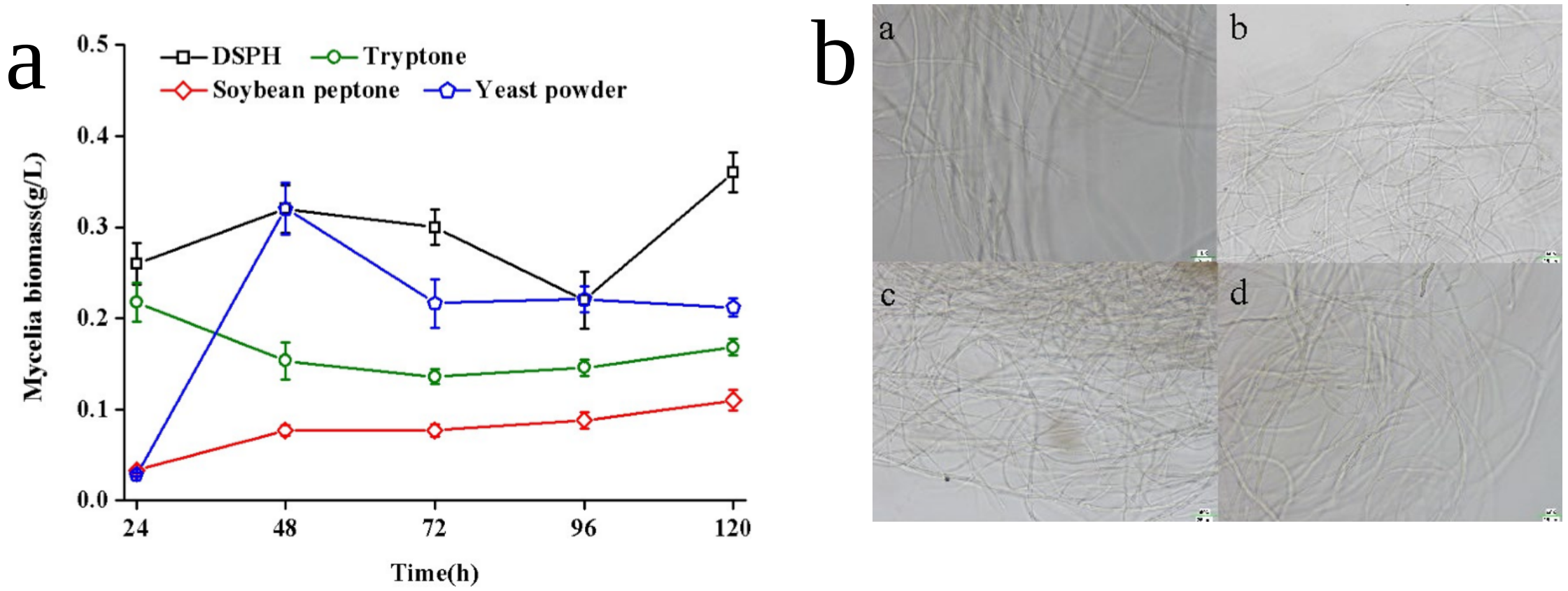


Fig. 10. The fermentation properties of four nitrogen source (a) and mycelia form comparation under microscope (b).

Results

Table 2 The economic analysis of different organic nitrogen sources was shown.

Organic nitrogen source	Total nitrogen content (%)	Purity	Specification (g)	Price (¥)	Unit price (¥/g)	Manufacturer
Tryptone	13.5±1.5	BR	500	95	0.19	Sinopharm Chemical Reagent Co., Ltd
Soybean peptone	≥8	BR	250	91	0.36	Sinopharm Chemical Reagent Co., Ltd
Yeast extract	10.9	BR	500	100	0.2	Thermo Fisher Scientific
Corn steep liquor	4	LR	540	130	0.24	Shanghai Fangqi Metrologic Instruments
Cottonseed powder	5	FMB	1000	200	0.2	Shaanxi Chengrui Biotechnology Co., Ltd
Malt extract	0.6	BR	25	60	2.4	Thermo Fisher Scientific
Casein	15±0.5	CP	250	85	0.34	Sinopharm Chemical Reagent Co., Ltd
DSPH	3.18±0.1	FMB	500	42	0.08	This study

Results

Table 3 The *Phelinus* fermentation effect of 6 kings of organic nitrogen source.

Nitrogen source selection	Additon (g/L)	Culture time(d)	Mycelia biomass (g/L)	Polysaccharides production (g/L)	Flavonoids Production (g/L)	Reference
Bran	5	8	6.886	0.020	0.007	Zheng, W., 2017.
Yeast extract	5	5	0.85	0.102	-	Yang, C.F., ect, 2016.
Soybean meal	5.7	7	-	0.177	0.11	Wang, S.N., ect, 2016.
Tryptone	5	7	5	-	0.33	Lin, Q.Y., ect, 2018.
Yeast extract	5	14	1.1	-	0.30	Zheng, F., ect, 2017.
DSPH	5	5	0.36	0.376	0.22	This study

Conclusions

1. This study innovatively used **DSPH** as an alternative nitrogen source to improve a series of active metabolites of the medicinal fungus *P. baumii* MK818502.

2. By single factor optimization experiment the optimal DSPH content was **32 g/L**, c ulture temperature was **26 ℃**, pH was **7** and rotation speed was **120 rpm**.

3. The maximum values mycelia biomass was **0.707 g/L**, extracellular polysaccharide s was **1.54 g/L** and extracellular flavonoids was **0.078 g/L**. The kinetic models coul d be used for reference in the industrial production of polysaccharides flavonoids a nd bath feeding substrates.

4. By contrast with other nitrogen sources, the DSPH biomass accumulated to **0.36 g/ L**, the cost of production was **0.08 ¥/g** and produced **0.376 g/L** extracellular polysa ccharides which was maximum in *P. baumii* fermentation comparison.

Acknowledgments

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Thank you for your attention