

RESEARCH

Open Access



# Enzymatic process optimization for the in vitro production of isoprene from mevalonate

Tao Cheng<sup>1,3†</sup>, Hui Liu<sup>1†</sup>, Huibin Zou<sup>1,2\*</sup>, Ningning Chen<sup>2</sup>, Mengxun Shi<sup>2</sup>, Congxia Xie<sup>3</sup>, Guang Zhao<sup>1\*</sup> and Mo Xian<sup>1\*</sup>

## Abstract

**Background:** As an important bulk chemical for synthetic rubber, isoprene can be biosynthesized by robust microbes. But rational engineering and optimization are often demanded to make the in vivo process feasible due to the complexities of cellular metabolism. Alternative synthetic biochemistry strategies are in fast development to produce isoprene or isoprenoids in vitro.

**Results:** This study set up an in vitro enzyme synthetic chemistry process using 5 enzymes in the lower mevalonate pathway to produce isoprene from mevalonate. We found the level and ratio of individual enzymes would significantly affect the efficiency of the whole system. The optimized process using 10 balanced enzyme units (5.0  $\mu\text{M}$  of MVK, PMK, MVD; 10.0  $\mu\text{M}$  of IDI, 80.0  $\mu\text{M}$  of ISPS) could produce 6323.5  $\mu\text{mol/L/h}$  (430 mg/L/h) isoprene in a 2 ml in vitro system. In a scale up process (50 ml) only using 1 balanced enzyme unit (0.5  $\mu\text{M}$  of MVK, PMK, MVD; 1.0  $\mu\text{M}$  of IDI, 8.0  $\mu\text{M}$  of ISPS), the system could produce 302 mg/L isoprene in 40 h, which showed higher production rate and longer reaction phase with comparison of the in vivo control.

**Conclusions:** By optimizing the enzyme levels of lower MVA pathway, synthetic biochemistry methods could be set up for the enzymatic production of isoprene or isoprenoids from mevalonate.

**Keywords:** Mevalonate, Isoprene, Synthetic biochemistry, Isoprenoids, Bio-based chemicals, Enzymatic process

## Background

Commodity chemicals are traditionally produced from petroleum via the energy-intensive chemical methods. Alternative process that does not rely on the petroleum resources and chemo-process is under fast development for chemical production, using the advanced tools of synthetic biology and metabolic engineering [1, 2]. Recently, isoprene and a variety of isoprenoids can be produced from renewable feedstock through engineered microbes [3–7]. The general strategy is engineering the whole isoprenoid biosynthesis pathways in the chassis strains [6], and the mevalonate (MVA) pathway other than the methylerythritol 4-phosphate (MEP) pathway is usually

selected to construct robust strains with higher production titers [8].

However, the biosynthesis of isoprene and isoprenoids by the cell-based strategy faced one major bottleneck: the nutrient limitation and the accumulation of toxic intermediates or products may lead to cell growth inhibition and limited yields and titers; thus rational engineering and optimization are often demanded to make the process economically feasible [9–12]. Corresponding synthetic biochemistry tool has also been demonstrated to overcome the major bottleneck of the cell-based strategy, especially in rational optimization of synthetic multi-enzyme pathways [13]. The emergence of cell-free system has several advantages over the cell-base system: (1) process can be operated continuously; (2) can synthesize hazard products which are toxic towards living cell system; (3) enzyme can be quantitatively and biochemically adjusted to optimize flux through the metabolic pathways; (4) reduce the operation (like gas-stripping) and

\*Correspondence: huibinzou@hotmail.com; zhaoguang@qibebt.ac.cn; xianmo@qibebt.ac.cn

<sup>†</sup>Tao Cheng and Hui Liu contributed equally to the work

<sup>1</sup> CAS Key Laboratory of Bio-based Materials, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, No. 189 Songling Road, Laoshan District, Qingdao 266101, China  
Full list of author information is available at the end of the article

purification requirements; (5) higher yields without competing cellular pathways. In case of isoprene biosynthesis, a recent cell-free approach for the conversion of the glycolysis intermediate phosphoenolpyruvate into isoprene at nearly 100% molar yield was promoted [14]. The in vitro system involves enzymes through the full MVA pathway. While challenges also exist for the synthetic biochemistry platform, as the cofactors of ATP, NADPH, and acetyl-CoA need to be balanced in the complex MVA pathway: NADPH and acetyl-CoA are involved in the upper MVA pathway (from pyruvate to MVA), ATP is involved in the lower MVA pathway (from MVA to isoprene). Thus additional enzymes are needed to balance the supply/consumption of these co-factors which increased the total enzymes of 12 in the complex in vitro system for bioisoprene [13, 14].

This study focused on implementing an enzymatic process only consisting of lower MVA pathway for the biosynthesis of isoprene directly from MVA. In the demonstrated system, the cofactors of NADPH and acetyl-CoA are not involved, which decreases the needs of additional enzymes to balance these factors. Moreover, the enzyme activities and quantities of the simplified system (only involves 5 enzymes) can be easily and precisely

adjusted to optimize flux through the biochemical steps, which would improve the conversion efficiency comparing to the current in vitro platform for bioisoprene.

## Results and discussion

### Purification of the enzymes in lower MVA pathway

In this study, four biosynthetic enzymes of lower MVA pathway and isoprene synthase were individually prepared by recombinant *E. coli* strains (Table 1) and purified before in vitro production. Through these enzymes mevalonate can be converted to dimethylallyl pyrophosphate (DMAPP) by primary mevalonate kinase (MVK, EC 2.7.1.36) and secondary phosphorylation (phosphomevalonate kinase, PMK, EC 2.7.4.2), decarboxylation (diphosphomevalonate decarboxylase, MVD, EC 4.1.1.33) and isomerization (isopentenyl diphosphate isomerase, IDI, EC 5.3.3.2), then the isoprene synthase (ISPS, EC 4.2.3.27) catalyzes the formation of isoprene from DMAPP. For each molecular of isoprene from MVA, 3 ATP is needed and NADPH is not demanded for the in vitro bioconversion, which is different with the in vivo system from pyruvate [14, 15]. In order to prevent the formation of inclusion body and ensure correct enzyme folding, each enzyme was purified and checked

**Table 1 Bacterial strains and plasmids used in this study**

| Strain/plasmid/primer    | Relevant genotype/property/sequence  | Source/reference |
|--------------------------|--|------------------|
| Strains                  |  |                  |
| <i>E. coli</i> BL21(DE3) | F <sup>-</sup> <i>ompThsdS<sub>B</sub>(r<sub>B</sub><sup>-</sup>m<sub>B</sub><sup>-</sup>) gal dcm rne131 (DE3)</i>          | Invitrogen       |
| MVK producer             | BL21(DE3)/pET-ERG12  | This study       |
| MVD producer             | BL21(DE3)/pET-ERG8   | This study       |
| PMK producer             | BL21(DE3)/pET-ERG19  | This study       |
| IDI producer             | BL21(DE3)/pET-IDI  | This study       |
| ISPS producer            | BL21(DE3)/pET-ISPS   | This study       |
| Plasmids                 |  |                  |
| pYJM14                   | pTrcHis2B derivative carrying genes <i>ERG8</i> , <i>ERG12</i> , <i>ERG19</i> and <i>IDI</i> , Trc promoter, Ap <sup>R</sup> | [16]             |
| pET-ERG12                | pET30a(+) derivative carrying genes gene <i>ERG12</i> , T7 promoter, Kan <sup>R</sup>  | This study       |
| pET-ERG8                 | pET30a(+) derivative carrying genes gene <i>ERG8</i> , T7 promoter, Kan <sup>R</sup>   | This study       |
| pET-ERG19                | pET30a(+) derivative carrying genes gene <i>ERG19</i> , T7 promoter, Kan <sup>R</sup>  | This study       |
| pET-IDI                  | pET30a(+) derivative carrying genes gene <i>IDI</i> , T7 promoter, Kan <sup>R</sup>  | This study       |
| pET-ISPS                 | pET30a(+) derivative carrying genes gene <i>ISPS</i> , T7 promoter, Kan <sup>R</sup>   | This study       |
| pACYDuet-1               | pACYDuet-1 derivative carrying genes gene <i>ISPS</i> , T7 promoter, Cm <sup>R</sup>   | This study       |
| Primers                  |  |                  |
| ERG12_F                  | 5'-CCCAAGCTTGGTCATTACCGTTCTTAAC TTC-3'   |                  |
| ERG12_R                  | 5'-CCGCTCGAGTTATGAAGTCCATGGTAAAT-3'  |                  |
| ERG8_F                   | 5'-CCGGAATTCTCAGAGTTGAGAGCCTTCAG-3'  |                  |
| ERG8_R                   | 5'-CCGCTCGAGTTATTTATCAAGATAAGTTT-3'  |                  |
| ERG19_F                  | 5'-CGCGGATCCACCGTTTACACAGCATCCGT-3'  |                  |
| ERG19_R                  | 5'-CCGCTCGAGTTATTCCTTTGGTAGACCAG-3'  |                  |
| IDI_F                    | 5'-CGCGGATCCACTGCCACAACAATAGTAT-3'   |                  |
| IDI_R                    | 5'-CCGCTCGAGTTATAGCATTCTATGAATTT-3'  |                  |

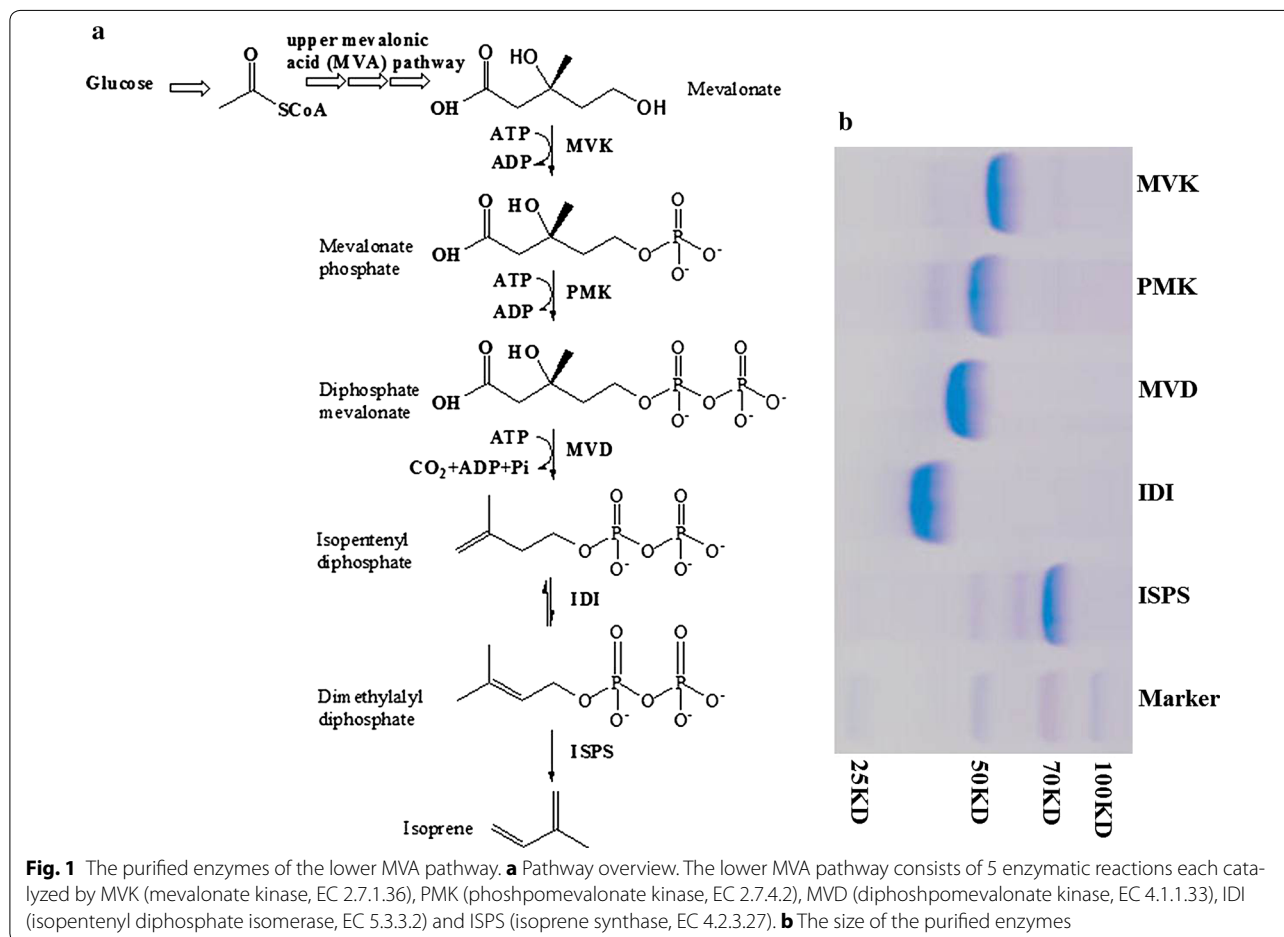
their molecular size by SDS-page before enzymatic assay experiments. We found that keeping the cultures at 20 °C after IPTG induction in the fermentation process helped to improve the yield of soluble recombinant enzymes. The purity and molecular size of the purified enzymes can be seen in Fig. 1.

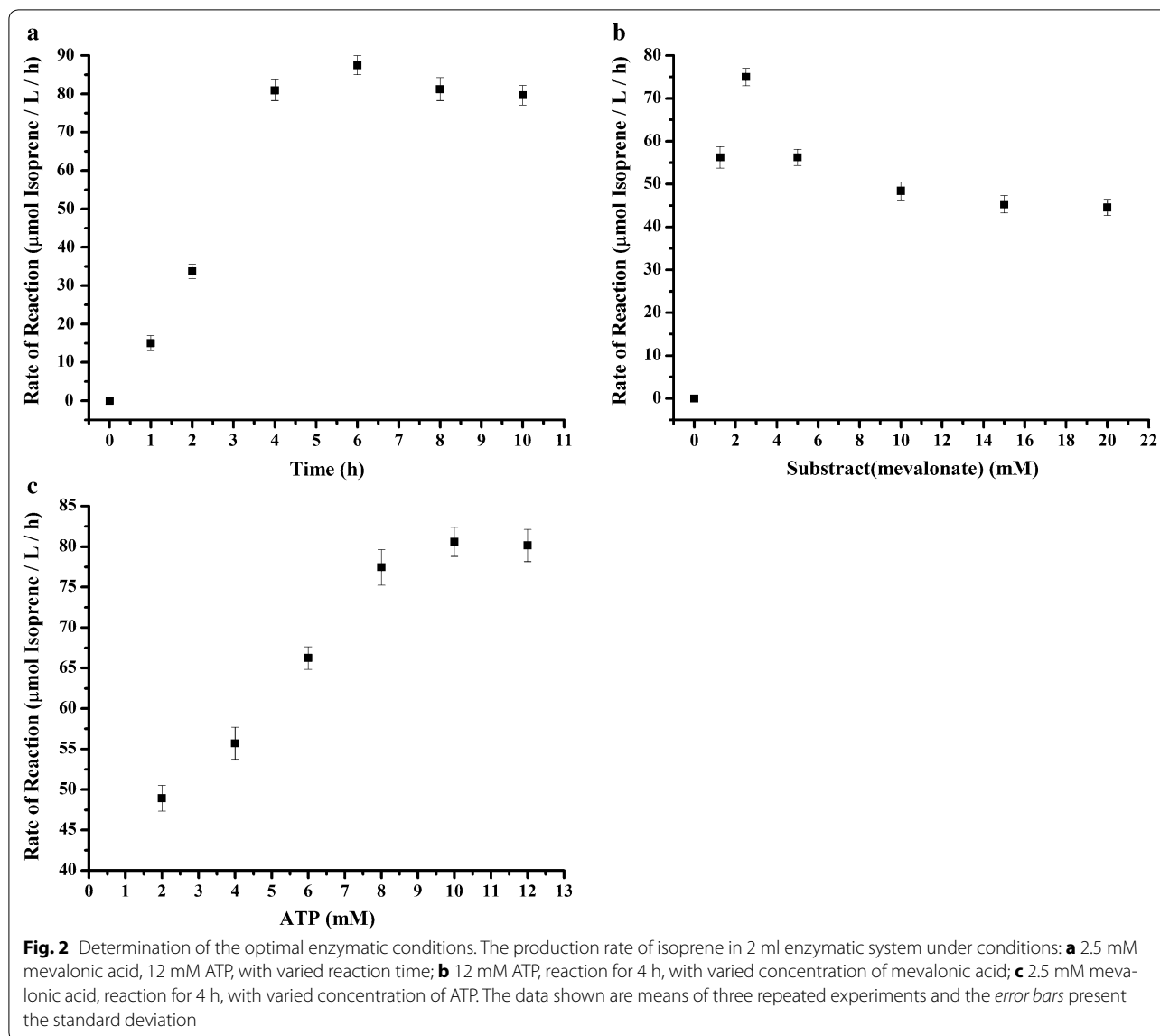
### In vitro production of isoprene from mevalonate

With the purified enzymes, we further tested whether the lower mevalonate pathway can be reconstituted in vitro by mixing of substrate and ATP with the purified enzymes. The addition of ATP is compulsory as three ATP are used in the phosphorylation and decarboxylation of mevalonate to IPP (Fig. 1). In the 2 ml in vitro system with purified enzymes (0.5 μM each), substrate (2.5 mM) and ATP (12 mM), we took samples and analyzed total isoprene at different time points and calculate the rate of reaction (total isoprene/L/h) at the different time points. The results showed that the maximum isoprene production (76.5 μmol/L/h, 5.2 mg/L/h) occurred at 4 h after the addition of ATP and mevalonate (Fig. 2a),

similar with the time course curve of in vitro isoprene production from PEP in the previous study [14].

We next tested the optimum mevalonate/ATP ratio in the 2 ml in vitro system. The optimum concentration of mevalonate (substrate) was analyzed by starting with a fixed amount of ATP (12 mM) and sequentially increasing amount of mevalonate (Fig. 2b). The results showed that the maximum isoprene production was achieved when 2.5 mM mevalonate was added in the system, and the isoprene production was decreased when the initial concentration of mevalonate was over 2.5 mM. We also tested the optimum concentration of ATP with fixed amount of mevalonate (2.5 mM). The results showed that supplementation of 10 mM ATP resulted in the maximum isoprene production (Fig. 2c). The production of isoprene was increasing with the increasing of ATP from 2 to 12 mM, it is suggested that the lower pathway of MVA was inhibited when the concentration of substrate of mevalonate was higher than 2.5 mM. The optimum ratio for substrate (mevalonate) and cofactors (ATP) is around 1:4, which is excess to the theoretical



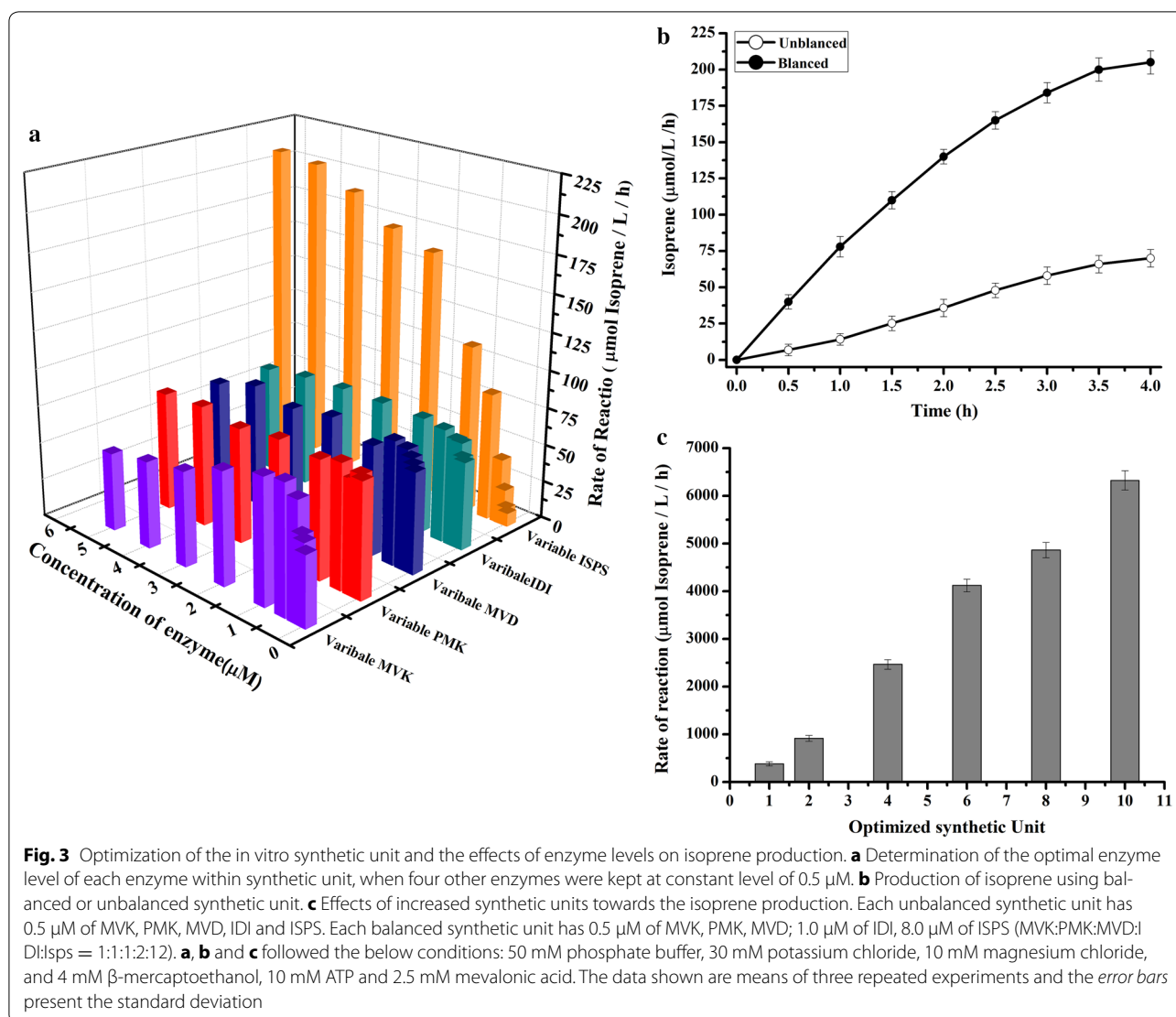


ATP consumption (1:3) in the lower mevalonate pathway (Fig. 1). The excess addition of ATP is similar with previous study when acetyl-CoA was utilized as substrate for the *in vitro* production of isoprene [14].

#### Quantitatively balancing enzyme levels to maximum the isoprene production

In the *in vitro* isoprene biosynthesis system, the enzyme quantity can be precisely adjusted to optimize the flux through the biochemical steps comparing with the isoprenoids biosynthesis using living cells, in which promoter induction is often highly cooperative and fine control is difficult [11]. To quantitatively construct balanced *in vitro* system, we firstly screened the bottleneck enzymes which significantly affect the isoprene

production. The effects of enzyme levels towards isoprene production were tested by varying the levels between 0.02 and 5  $\mu$ M with the constant levels of other four enzymes at 0.5  $\mu$ M (Fig. 3a) in the 2 ml *in vitro* system. The results showed that the enzymes had different effects towards the isoprene production. PMK and MVD were not belonging to the bottleneck enzymes in the pathway, as their levels did not significantly influence the isoprene production. For the enzymes of MVK and IDI, their increasing levels generated a 30–90% increase in isoprene production: MVK reached the maximum isoprene production at the level of 0.5  $\mu$ M while IDI reached the maximum isoprene production at 1.0  $\mu$ M (Fig. 3a). The last enzyme of ISPS, which catalyzes the production of isoprene from DMAPP, belonged to the bottleneck



enzyme of this in vitro system, as its increasing levels from 0.02 to 5  $\mu\text{M}$  gave a 30-fold increase in isoprene production. The results indicated that IDI and ISPS were two bottleneck enzymes in the in vitro production of isoprene from mevalonate, and their levels needed to be adjusted to balance the in vitro system. From the data of Fig. 1a, we could deduce that the optimized molarity ratio of MVK: PMK: MVD: IDI: ISPS was 1:1:1:2:16. One such balanced synthetic unit (0.5  $\mu\text{M}$  of MVK, PMK, MVD; 1.0  $\mu\text{M}$  of IDI, 8.0  $\mu\text{M}$  of ISPS) could produce up to 382.4  $\mu\text{mol/L/h}$  (26 mg/L/h) isoprene, which is nearly five folds of the isoprene production by the unbalanced synthetic unit (0.5  $\mu\text{M}$  of each enzyme, Fig. 3b). Moreover, when multiple synthetic unit is supplemented in the 2 ml in vitro system, the production efficiency of isoprene could be significantly improved, 10 balanced synthetic

units (5.0  $\mu\text{M}$  of MVK, PMK, MVD; 10.0  $\mu\text{M}$  of IDI, 80.0  $\mu\text{M}$  of ISPS) would increase the isoprene production to 6323.5  $\mu\text{mol/L/h}$  (430 mg/L/h) (Fig. 3c).

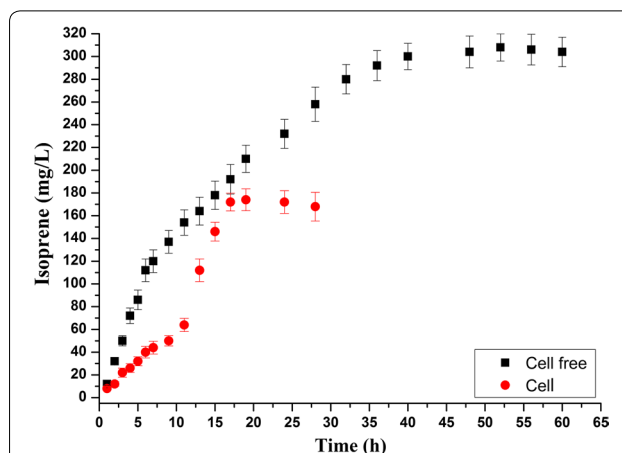
Our results demonstrated that balancing of enzyme levels could significantly increase the in vitro production of isoprene, which followed the results of in vivo studies that the balanced expression levels of heterologous enzymes is a key determinant in optimizing isoprenoid production [10, 11]. The in vivo studies aim to balance the heterologous pathways to reduce the growth inhibition effects towards the microbial hosts while the in vitro studies more focus on overcoming the limiting biochemical steps in heterologous flux towards the objective products. For example, we have noted that the enzymes levels of MVK did not apparently affect the in vitro production of isoprene (Fig. 3a) and they were kept at minimum level

(0.5  $\mu\text{M}$ ) in our balanced synthetic unit. While in the *in vivo* studies for isoprene [3, 16] and isoprenoids biosynthesis [11], the expression of MVK were adjusted at higher levels, in which MVK was believed to be a key enzyme and was expressed by stronger promoters than PMK and MVD. We hypothesized that the *in vivo* system has specific mechanism to adjust the intra- and extracellular levels of mevalonate (MVA), and higher level of MVK helps to convert the intracellular MVA before it is transported off the cell. The last enzyme ISPS were both found bottleneck enzyme in previous study [17] as well as in this study. From the data of this study, ISPS catalyzed the rate-limiting biochemical step in heterologous flux from DMAPP to the isoprene. The *in vivo* models also supported this conclusion, it was proved that lower expression level of key enzyme downstream the IPP/DMAPP will lead to the accumulation of C5 building blocks (IPP and DMAPP), and will inhibit normal cell growth [9].

Comparing with the previous *in vitro* experiment [14] which incorporated as much as 12 enzymes of upper and lower MVA pathways, this study demonstrated a simpler system which only involved 5 enzymes and their levels were precisely adjusted to optimize flux through the biochemical steps. After optimization, the isoprene production was significantly improved (from 214.5 to 6323.5  $\mu\text{mol/L/h}$ ) comparing with the previous *in vitro* experiment [14].

#### **In vitro and in vivo production of isoprene from MVA: a comparison**

We further set up a 50 ml *in vitro* system and compared its isoprene production with the *in vivo* flask fermentation (50 ml), from the same starting concentration of mevalonate substrate. The results showed that the *in vitro* model apparently had higher isoprene production rate than the *in vivo* model during the earlier 12 h of isoprene production (Fig. 4). The initial isoprene production rate is about 220.6  $\mu\text{mol/L/h}$  (15.0 mg/l/h, total 160 mg/l isoprene within 12 h), which is similar with the recent study for the *in vitro* isoprene production system [14]. Moreover, isoprene production remained longer for the *in vitro* system: from 20 to 40 h, the production of isoprene was suspended in the *in vivo* system, but was consistent in the *in vitro* system. Until 40 h, the production of isoprene from mevalonate by the *in vitro* system reached to 4442.4  $\mu\text{mol/L}$  (302.0 mg/l). From the comparison of the isoprene production by the *in vitro* and the *in vivo* models, we could deduce that the balanced *in vitro* enzyme system led to higher production rate and prolonged isoprene production. We estimated that the unbalanced intracellular enzymes levels, accumulated toxic intermediates, competitive consumption of co-factors may affect the isoprene production by the *in vivo* methods.



**Fig. 4** The comparison of isoprene production by 50 ml *in vitro* and *in vivo* systems. The concentration of MVK (0.5  $\mu\text{M}$ ) was similar in both *in vitro* (black) and *in vivo* (red) systems. The cell free *in vitro* system has 1 synthetic unit (0.5  $\mu\text{M}$  of MVK, PMK, MVD; 1.0  $\mu\text{M}$  of IDI, 8.0  $\mu\text{M}$  of ISPS) with 50 mM phosphate buffer, 30 mM potassium chloride, 10 mM magnesium chloride, and 4 mM  $\beta$ -mercaptoethanol. 50 ml culture of engineered *E. coli* (BL21(DE3)/pYJM14/pACY-ISPS, Table 1) which expressed the five enzymes of the lower MVA pathway was utilized as the *in vivo* control. Both systems was initiated by addition of 2.5 mM mevalonic acid and incubated at 30  $^{\circ}\text{C}$  in rotary shaker (180 rpm). Samples were taken to analyze their isoprene production at different time points. The data shown are means of three repeated experiments and the error bars present the standard deviation

## **Conclusion**

In this study, isoprene was produced from mevalonate by an optimized enzymatic process using the five recombinant enzymes of MVK, PMK, MVD, IDI and ISPS from the lower MVA pathway. Balancing and increasing the enzyme levels could apparently enhance isoprene production, indicated that the production rate could be further increased by raising the concentrations of the mevalonate and enzymes, which is hard to achieved by the *in vivo* approaches. When the balanced enzyme units were increased to ten, the proposed process would produce 6323.5  $\mu\text{mol/L/h}$  (430 mg/L/h in a 2 ml system) isoprene from mevalonate. Moreover, this study showed that the proposed process has the advantages of longer producing period of 40 h comparing with the controlled *in vivo* process. The improved production efficiency indicated that the proposed strategy is useful for the enzymatic production of isoprene or isoprenoids.

## **Methods**

### **Strains and plasmids**

Bacterial strains and plasmids used in this study were listed in Table 1. *Escherichia coli* strain DH5 $\alpha$  and BL21 (DE3) and *Saccharomyces cerevisiae* used in this study were purchased from Invitrogen. The *E. coli* DH5 $\alpha$  strain and *E. coli* BL21 (DE3) were used for plasmids

preparation and for protein overexpression respectively. *S. cerevisiae* was used for gene for cloning. *E. coli* DH5 $\alpha$  and *E. coli* BL21 (DE3) were cultured in Luria–Bertani (LB) broth in construction of strains and plasmids. *S. cerevisiae* was cultured in YPD medium. Antibiotics were added at final concentration of 50  $\mu$ g/mL for kanamycin, 34  $\mu$ g/mL for chloramphenicol and 100  $\mu$ g/mL for ampicillin when necessary.

#### Protein preparation and purification

All PCRs were done using PrimerSTAR Max DNA polymerase (TAKARA, Dalian, China). Four genes of the enzymes MVK, PMK, MVD, IDI were amplified from *S. cerevisiae* genome (obtained from ATCC201508D) and cloned into the plasmid pET-30a(+). The plasmid pET-ERG12 was constructed by cloning the *ERG12* gene (for MVK) of *S. cerevisiae* into *Hind*III and *Xho*I sites of vector pET-30a(+) with primers ERG12\_F and ERG12\_R. The plasmid pET-ERG8 was constructed by cloning the *ERG8* gene (for PMK) from *S. cerevisiae* into *Eco*RI and *Xho*I sites of vector pET-30a(+) with primers ERG8\_F and ERG8\_R. The plasmid pET-ERG19 was constructed by cloning the *ERG19* gene (for MVD) from *S. cerevisiae* into *Bam*HI and *Xho*I sites of vector pET-30a(+) with primers ERG19\_F and ERG19\_R. The fourth gene for enzyme IDI was amplified by PCR using primers IDI\_F and IDI\_R and cloned into *Bam*HI and *Xho*I sites of vector pET-30a(+). The resulting plasmid was named pET-IDI. The isoprene synthase (ISPS) from poplar was synthesized after code optimization and digested with enzymes *Bam*HI and *Xho*I, then ligated into the pET-30a(+), the resulting plasmid was named pET-ISPS (Table 1).

Individual recombinant enzymes were extracted and purified from the strains of *E. coli* BL21(DE3) harboring the relevant plasmids. The cultures were incubated in 200 ml LB medium with 50  $\mu$ g/ml Kanamycin at 37 °C until the OD600 reached 0.6–0.8. The cultures were added 0.2 mM IPTG for induction and were cooled to 20 °C for protein expression. After further growth at 20 °C for 12 h, cells were harvested by centrifugation at 8000g and suspended in 10 mL 50 mM phosphate buffer (pH7.4) containing 4 mM  $\beta$ -mercaptoethanol. The suspension was lysed by sonication (at 60% output for 3 s pulses with 3 s intervals between each cycle) for 40 min at 4 °C with tube jacketed in wet ice and centrifuged at 18,000g for 10 min at 4 °C. The supernate filtered by 0.22  $\mu$ m PALL filter was added into the Nickel Column, which was washed by 10 ml water and 10 ml binding buffer in order to ensure that the column was equilibrated, and then the protein containing 6 His-tag was able to specifically bind to nickel column. Unbound protein was washed out with 10 ml washing buffer 1, then

washing Buffer 2 was used to wash any nonspecific binding protein. Elution buffer was added to wash the specific protein with collection of 10 ml fractions. The column was then re-equilibrated with buffer. Protein concentrations were measured with BCA protein assay kit using a spectrophotometer. Recombinant enzymes were stored at –80 °C after flash freezing in liquid nitrogen.

#### In vitro reaction system

The reaction system was performed as previously described [18] to ensure the correct concentration of individually enzyme. For the demonstrated assay, a variety levels of each enzyme component and ATP were added to the reaction buffer which contain 50 mM potassium phosphate, 30 mM potassium chloride, 10 mM magnesium chloride, and 4 mM  $\beta$ -mercaptoethanol. The reaction was initiated by addition of 2.5 mM mevalonic acid which was made by the saponification of mevalonolactone with KOH at 1.05:1 (vol:vol) KOH:mevalonolactone for 30 min at 37 °C, and then incubated at 30 °C for 4 h. To confirm isoprene had accumulated in the 2 ml reaction system, 0.2 ml of the headspace gas of sealed 10 ml vial were analyzed by gas chromatography using Agilent 7890B GC (Agilent, American) equipped with a flame ionization detector and a Agilent HP-INNOWOX column, designed to detect short-chain hydrocarbons. Amounts of isoprene produced in the recombinant system were calculated by comparison with an isoprene standard (Aladdin, China).

#### Gas chromatography (GC) analysis of isoprene

1 ml of off-gas samples from the headspace of the fermentor were analyzed as described earlier [16] using a GC (Agilent 7890A, America) equipped with a flame ionization detector (FID) and a HP-INNOWAX column (30 m  $\times$  320  $\mu$ m  $\times$  8  $\mu$ m). N<sub>2</sub> was used as carrier gas with a linear velocity of 1 ml/min. The product was characterized by direct comparison with standard isoprene (TCI-EP, Tokyo, Japan). The peak area was converted to isoprene concentration by comparing with a standard curve plotted with a set of known concentration of isoprene. Then isoprene accumulation was measured every 30 min by GC.

#### The comparison of production of isoprene in vitro and in vivo

To compare isoprene production in shake flasks (in vivo) and in vitro, the engineered strain *E. coli* BL21(DE3)/pYJM14/pACY-ISPS (Table 1) were cultured in 500 ml sealed glass flasks containing 50 ml of M9 medium supplemented with 10 g/L glucose and 0.5 g/L yeast extract, 34  $\mu$ g/mL chloramphenicol, and 100  $\mu$ g/mL Ampicillin. IPTG was added to the medium when the cell density

OD<sub>600</sub> reached to 0.6 and the cultures were performed at 30 °C in rotary shaker (180 rpm). For isoprene production, the substrate 2.5 mM mevalonate was added after the cell was induced and cultivated at 30 °C for 4 and 16 h respectively. During the cultivation, samples of both the flask headspace and the culture were taken at multiple times points.

To set up similar enzyme levels in the in vitro system comparing to the in vivo control. The intracellular MVK concentration of the in vivo strain was firstly quantified using Quantity One Software (Biorad) described previously [19]. When OD<sub>600</sub> reached 0.6, intracellular MVK concentration was about 0.4 μM. According to the optimized molarity ration for the in vitro system, 0.4 μM of MVK, PMK, MVD, 0.8 μM of IDI, 6.4 μM of ISPS was added in the 50 ml in vitro reaction system. 10 mM ATP, 50 mM potassium phosphate, 30 mM potassium chloride, 10 mM magnesium chloride, and 4 mM β-mercaptoethanol were also added in the reaction system. The reaction was initiated by addition of 2.5 mM mevalonic acid which was made by the saponification of mevalonolactone with KOH at 1.05:1 (vol:vol) KOH:mevalonolactone for 30 min at 37 °C, and then incubated at 30 °C in rotary shaker (180 rpm). During the cultivation, samples of both the flask headspace and the culture were taken at multiple times points.

#### Abbreviations

MVA pathway: mevalonate pathway; MEP pathway: methylerythritol 4-phosphate pathway; MVK: mevalonate kinase; PMK: phosphomevalonate kinase; MVD: diphosphomevalonate decarboxylase; IDI: isopentenyl diphosphate isomerase; ISPS: isoprene synthase; IPTG: isopropyl-β-d-thiogalactoside; GC: gas chromatography.

#### Authors' contributions

TC and HL conceived of the study, participated in its design, carried out the process control studies and drafted the manuscript. MS and NC participated in the coordination of this study, contributed to the data analysis and the process control studies. GZ and CX participated in its design and helped to draft the manuscript. MX and HZ conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

#### Author details

<sup>1</sup> CAS Key Laboratory of Bio-based Materials, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, No. 189 Songling Road, Laoshan District, Qingdao 266101, China. <sup>2</sup> College of Chemical Engineering, Qingdao University of Science and Technology, Qingdao 266042, China. <sup>3</sup> State Key Laboratory Base of Eco-Chemical Engineering, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China.

#### Acknowledgements

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

#### Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

#### Ethics approval and consent to participate

Not applicable. The manuscript does not report data from humans or animals.

#### Funding

The present study was supported by Shandong Province Natural Science Foundation (ZR2015BM011), Technology Development Project of Shandong Province (2016GSF121013), National Natural Science Foundation (21106170, 21376129, 21572242, 31670493).

Received: 26 September 2016 Accepted: 27 December 2016

Published online: 09 January 2017

#### References

- Rabinovitch-Deere CA, Oliver JWK, Rodriguez GM, Atsumi S. Synthetic biology and metabolic engineering approaches to produce biofuels. *Chem Rev*. 2013;113(7):4611–32.
- Keasling JD. Synthetic biology and the development of tools for metabolic engineering. *Metab Eng*. 2012;14(3):189–95.
- Whited GM, Feher FJ, Benko DA. Development of a gas-phase bioprocess for isoprene-monomer production using metabolic pathway engineering. *Ind Biotechnol*. 2010;6(3):152–63.
- Chang MCY, Keasling JD. Production of isoprenoid pharmaceuticals by engineered microbes. *Nat Chem Biol*. 2006;2(12):674–81.
- George KW, Chen A, Jain A, Bath TS, Baidoo EEK, Wang G, Adams PD, Petzold CJ, Keasling JD, Lee TS. Correlation analysis of targeted proteins and metabolites to assess and engineer microbial isopentenol production. *Biotechnol Bioeng*. 2014;111(8):1648–58.
- Gronenberg LS, Marcheschi RJ, Liao JC. Next generation biofuel engineering in prokaryotes. *Curr Opin Chem Biol*. 2013;17(3):462–71.
- Zhang H, Liu Q, Cao Y, Feng X, Zheng Y, Zou H, Liu H, Yang J, Mo X. Microbial production of sabinene—a new terpene-based precursor of advanced biofuel. *Microb Cell Fact*. 2014;13(1):452–7.
- Immethun CM, Hoynes-O'Connor AG, Balassy A, Moon TS. Microbial production of isoprenoids enabled by synthetic biology. *Front Microbiol*. 2013;4:75.
- Martin VJ, Pitera DJ, Withers ST, Newman JD, Keasling JD. Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. *Nat Biotechnol*. 2003;21(7):796–802.
- Pitera DJ, Paddon CJ, Newman JD, Keasling JD. Balancing a heterologous mevalonate pathway for improved isoprenoid production in *Escherichia coli*. *Metab Eng*. 2007;9(2):193–207.
- Ajikumar PK, Xiao W-H, Tyo KEJ, Wang Y, Simeon F, Leonard E, Mucha O, Phon TH, Pfeifer B, Stephanopoulos G. Isoprenoid pathway optimization for taxol precursor overproduction in *Escherichia coli*. *Science*. 2010;330(6000):70–4.
- Dueber JE, Wu GC, Malmirchegini GR, Moon TS, Petzold CJ, Ullal AV, Prather KL, Keasling JD. Synthetic protein scaffolds provide modular control over metabolic flux. *Nat Biotechnol*. 2009;27(8):753–9.
- Hodgman CE, Jewett MC. Cell-free synthetic biology: thinking outside the cell. *Metab Eng*. 2012;14(3):261–9.
- Korman TP, Sahachartsiri B, Li D, Vinokur JM, Eisenberg D, Bowie JU. A synthetic biochemistry system for the in vitro production of isoprene from glycolysis intermediates. *Protein Sci*. 2014;23(5):576–85.
- Opgenorth PH, Korman TP, Bowie JU. A synthetic biochemistry molecular purge valve module that maintains redox balance. *Nat Commun*. 2014;17(5):4113.
- Yang J, Xian M, Su S, Zhao G, Nie Q, Jiang X, Zheng Y, Liu W. Enhancing production of bio-isoprene using hybrid MVA pathway and isoprene synthase in *E. coli*. *PLoS ONE*. 2012;7(4):e33509.
- Ilmén M, Oja M, Huuskonen A, Lee S, Ruohonen L, Jung S. Identification of novel isoprene synthases through genome mining and expression in *Escherichia coli*. *Metab Eng*. 2015;31:153–62.
- Yu X, Liu T, Zhu F, Khosla C. In vitro reconstitution and steady-state analysis of the fatty acid synthase from *Escherichia coli*. *Proc Natl Acad Sci USA*. 2015;108(46):18643–8.
- Jin K, Peel AL, Mao XO, Xie L, Cottrell BA, Henshall DC, Greenberg DA. Increased hippocampal neurogenesis in Alzheimer's disease. *Proc Natl Acad Sci USA*. 2004;101:343–7.