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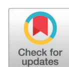

Original Article

# Biotransformation of Isobutyraldehyde to Isobutanol by an Engineered *Escherichia coli* Strain

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**Abstract**

**Introduction**: Biotransformation process has been used in various industries due to its ability to produce valuable chemicals and address environmental concerns. Propylene hydroformylation is a process in which n-butyraldehyde and isobutyraldehyde are produced. N-butyraldehyde is a high valuable chemical with many industrial applications, while isobutyraldehyde produced as a by-product is an environmental pollutant. This study offers a biotechnological approach for conversion of isobutyraldehyde into a high-value substance. An engineered strain of *Escherichia coli* was developed by genomic insertion of alcohol-dehydrogenase gene (*adhA*) from *Lactococcus lactis* which can convert isobutyraldehyde into isobutanol.

**Materials and Methods:** The *adhA* gene was engineered to substitute some of its amino acids to result in a more efficient enzyme. Engineered gene was synthesized and introduced into *E. coli* genome to develop recombinant *E. coli* EG-296 strain. In addition, by using the Qualiteck-4 software, 16 well-defined experiments ($L_{16}$ Orthogonal array) with two levels of seven variable parameters were used to optimize the process efficiency.

**Results:** The findings of this study revealed that the *E. coli* strain EG-296 is capable of converting isobutyraldehyde into isobutanol. The optimization results showed that optimum medium composition for the highest isobutanol production were 10 g/L glucose or glycerol as carbon source, 10 g/L NH4CL as nitrogen source, mid-log of inoculum age, and 1% inoculum volume in 25ml medium. After optimization, 560 mg/L isobutanol was produced from 600 mg/L isobutyraldehyde with 91% yield.

**Conclusions**: Recombinant *E. coli* strain with a relatively optimum medium can be used to remove isobutyraldehyde in refineries or other industries producing this chemical as a by-product.

**Keywords:** Biotransformation, Isobutyraldehyde, Recombinant *Escherichia coli*, Isobutanol, Optimization

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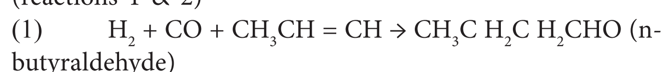
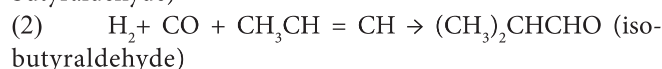
## Introduction

Biotransformation is the catalytic conversion of a chemical compound to another using biological systems.[1] The catalyst could be a whole cell, cell extract or enzymes.[2] There are numerous biotransformation processes in the nature of which many are now well-known. Biotransformation has been used to improve industrial processes and to produce biotechnological products[3] With the advent and progression of biotechnology approaches, biotransformation has found much more consideration[4] In addition, the limitation of global primary chemical resources as well as environmental concerns has led to the greater attention to biotransformation processes.[3]

One of the most critical applications of biotransformation is the bioremediation of environmental pollutants.[5,6] The bioremediation through biotransformation not only remove pollutants from the environment in a biocompatible and low-cost way, but also may convert these pollutants into high economic value materials.[7,8] Biotransformation could be used to replace inappropriate chemical methods with more efficient biotechnological ones to make some industries more environmentally friendly. For example, in the hydroformylation of propylene (Oxo synthesis), a mixture of carbon monoxide and hydrogen is used to produce normal-butyraldehyde along with iso-butyraldehyde as a by-product (with the ratio of 10:1), through the following reactions (reactions 1 & 2)[9,10]



(1) $H_2 + CO + CH_3CH = CH \rightarrow CH_3C\ H_2C\ H_2CHO$ (n-butyraldehyde)



(2) $H_2 + CO + CH_3CH = CH \rightarrow (CH_3)_2CHCHO$ (iso-butyraldehyde)

Although normal-butyraldehyde is a high-value industrial

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material with several applications, isobutyraldehyde is a low-cost toxic substance that is known as an environmental pollutant due to its various destructive environmental effects. Therefore, the conversion of isobutyraldehyde into a high-economic value substance is of great interest.[11,12] In this case, biotransformation can be used to convert the isobutyraldehyde into isobutanol, probably through developing bacterial strains capable of converting isobutyraldehyde into isobutanol. In the present study, we aimed to use metabolic and genetic engineering techniques to convert isobutyraldehyde into isobutanol through the strain development approach. Accordingly, the engineered alcohol-dehydrogenase gene (*adhA*) from *Lactococcus lactis* was introduced into the *E. coli* genome to develop a new recombinant *E. coli* EG-296 strain to consume isobutyraldehyde and produce isobutanol. A previous study[13] revealed that AdhA enzyme have the highest isobutyraldehyde reductase activity. In another study, Bastian et al[14] have substituted some of the amino acids of this enzyme to increase its efficiency, and these changes have also been applied. In addition, the Taguchi experimental design was used to optimize the isobutanol production and evaluate the efficiency of the production.

## Materials and Methods

### Bacterial Strains, Plasmids and Growth Condition

*Escherichia coli* XL-1 blue and *E. coli* BW25113 (Thermofisher Scientific, Waltham, Massachusetts, United States) strains were used as the cloning and final host, respectively. The pTZ57R/T and pUC19 plasmids (Thermofisher Scientific, Waltham, Massachusetts, United States) were used as cloning vectors and five primer pairs (Table 1) were used for PCR amplification and integration confirmation.

Quick and Easy *E. coli* Gene Deletion Kit (Gene Bridges, Heidelberg, Germany) were used for homologous recombination. Luria Bertani (LB) and M9 media were used to grow the *E. coli* strain at 37°C. The LB agar containing 100 µg/mL Ampicillin, 0.5 mM IPTG, 20 µg/mL X-Gal, and 30 µg/mL kanamycin was used to screen the recombinant bacterial clones. The M9 medium containing $Na_2HPO_4$ (48 mM), $KH_2PO_4$ (22 mM), NaCl (10 mM), and $(NH_4)_2SO_4$ (30 mM) supplemented with 1ml/L of each of glucose (20%), proline (20 mg/mL), thiamine (1 mg/mL), $CaCl_2$ (0.1 M), and $MgSO_4$ (1 M) was used for cell growth and isobutanol production.

### *Adh* Cassette Construction

For the conversion of isobutyraldehyde into isobutanol, the *adhA* gene from *L. lactis* was used to engineer *E. coli* BW25113. For the integration of *adhA* gene into *E. coli* genome by homologous recombination, a gene cassette including homology arms (1000 kb left and right), a selection marker gene (kanamycin resistant gene), and *adhA* gene was designed and constructed in pUC19 sequentially.

Genomic DNA of *E. coli* BW25113 was used as the PCR template for the amplification of the left and right homology arms. The amplified laeft and right arms clonend into *pstI-HindIII* and *EcoRI*, *-sacI* restriction sites of the pUC19, respectively, to construct the pYH-183 recombinant plasmid. The kanamycin gene, as a selection marker, was originated from kanamycin cassette of *E. coli* Gene Deletion Kit (Gene Bridges). The *kanamycin resistance* gene was amplified and cloned into the *XbaI* restriction site of pYH-183 to result in pYH-184 plasmid. The sequence of *adhA* gene encoding alcohol-dehydrogenase was obtained from NCBI database. The *adhA* gene with leucine 264 to valine (L264V), isoleucine 212 to threonine (I212T), and tyrosine 50 to phenylalanine (Y50F) amino acid substitutions[14] was codon optimized, according to codon usage of *E. coli*, and synthesized by the Generay Biotech Company (Generay Biotech., China). The synthetic *adhA* gene under the control of the PLlacO1 was cloned into the *BamH*I site of pYH-184 to result in pYH-185 as the final plasmid containing adh cassette.

### Integration of the *Adh* Cassette into *Escherichia coli* Genome

The pYH-185 was linearized by the *NdeI* enzyme and transformed into *E. coli* by electroporation using the Quick and Easy *E. coli* Gene Deletion Kit (Gene Bridges). All protocols for electroporation and homologous recombination were followed according to the kit's instructions. The integration of the cassette into the *E. coli* genome ( into *adhE* gene locus) was confirmed by kanamycin resistance, PCR, and Gas chromatography–mass spectrometry (GC–MS) analysis.

### GC–MS Analysis

Transformed *E. coli* EG-296 was grown in the presence of isobutyraldehyde for 18-24 hours. Two milliliters of the culture were centrifuged for 3 minutes at 10 000 rpm. One mililiter of the supernatant was transferred into a 5 mL caped

**Table 1.** Primers Used in This Study

| Primer Number | Primer Sequence | Usage |
|---|---|---|
| 1 | TATATAAGAATTCCCAGTAACGATAAATTCAATCC | Forward primer for left arm |
| 2 | TATATAGGTACCAGTAAATCATCTGCTCGAATACG | Reverse primer for left arm |
| 3 | TATATACTGCAGTCAGTAGCGCTGTCTGGCAAC | Forward primer for right arm |
| 4 | TATATAAAGCTTCTCGAGGCTAATGCTGTCGGTTTATGG | Reverse primer for right arm |
| 5 | ATATATTCTAGAGCGAAGTTCCTATTCTCTAG | Forward primer for *kanamycin resistance* gene |
| 6 | ATATAATCTAGAAGGAAGTTCCTATACTTTCTAG | Reverse primer for *kanamycin resistance* gene |
| 7 | ATAATCGGATCCAATTGTGAGCGGATAAC | Forward primer for *adhA* gene |
| 8 | ATTATCGGATCCTTATTTAGTAAAATCAATGAC | Reverse primer for *adhA* gene |
| 9 | TTTAGGCGTTATACATCGTCG | Forward primer for cassette insertion confirmation |
| 10 | CTCCGTTTCATTTGAAAGG | Reverse primer for cassette insertion confirmation |

vial and firmly sealed. The vial was heated at 40°C for 1 hour and 0.2 mL of gas above the heated culture supernatant was injected into the GC mass spectrometer. The area under the peak was calculated by the ChemStation software. By plotting a horizontal line from the numerical value of the area below each pick graph and its collision with the standard curve, the concentration of isobutanol was calculated.

The isobutanol produced by recombinant *E. coli* strain was identified by GC–MS 7890b GC system (Agilent Technologies, Santa Clara, California, United States) with 7883B injector and autosampler (Agilent Technologies, Santa Clara, California, USA) and 5973 network mass selective detector Fluka. A DB-5 ms capillary column (30 m, 0.25 mm internal diameter, 0.25 µm film thickness; Agilent Technologies) was used with helium (1 mL/min) as the carrier gas. The oven temperature was programmed from 30℃ (2.6 minutes). The inlet and auxiliary temperatures were 180℃ and 280℃, respectively. Alcohol compounds were isolated by solvent extraction. Two milliliters of the broth culture supernatant were extracted with 150 mL GC standard grade toluene (Fluka), and a 2-µL sample was injected in the split injection mode with a 200:1 split ratio.

### Design of Experiment (DOE)

The Taguchi methodology was used to evaluate the cumulative effects of variables affecting the isobutyraldehyde biotransformation to isobutanol. Since biotransformation is a growth- associated process, seven factors (Table 2), including glucose and glycerol concentrations (as carbon sources), NH4Cl and yeast extract concentrations (as nitrogen sources), the volume and age of inoculum as well as the volume of culture media were evaluated. The Qualiteck-4 software was used for automatic experimental design and analyzing the results. Since these factors have positive and negative interactions depending on their levels, they are optimized on the basis of Orthogonal Arrays (OA) and Taguchi method. The OA obtained from the Taguchi method containing 16 experiments is shown in Table 3. To reduce the noise effect, the entire set of experiments were carried out three times. Furthermore, to determine the influence of a particular parameter on the production of isobutanol and to measure the robustness of control factors that reduce the variability in the product or process, the Signal-to-Noise ratio (S/N ratio) was conducted.[15] In this study, the S/N ratio was calculated by using the following equation 1:

$$S/N = -10^{*} \log (\Sigma (1/Y^{2})/n) \qquad 1$$

Y”is the signal (amount of isobutanol produced) and“n”is the number of repetitions in each experiment. In addition, analysis of variance (ANOVA) was used to study the Taguchi $L_{16}$ OA experiment data and the percentage contributed by each factor and its interactions.

**Table 2.** Factors and Their Levels Used in Taguchi’s OA Design for Isobutyraldehyde Conversion

| Name | Factor | Level 1 | Level 2 |
|---|---|---|---|
| A | Glucose | 10 (g/L) | 20 (g/L) |
| B | Glycerol | 10 (g/L) | 30 (g/L) |
| C | NH4Cl | 10 (g/L) | 20 (g/L) |
| D | Yeast extract | 2 (g/L) | 6 (g/L) |
| E | Inoculum age | Mid-Log | Stationary |
| F | Inoculum volume | 1% | 5% |
| G | Culture volume | 10 (mL) | 25 (mL) |

## Results

### Integration of Adh Cassette into *Escherichia coli* Chromosome

All segments of the adh cassette were cloned in pUC19 sequentially. The final plasmid, namely pYH-185 containing the adh cassette was linearized by *NdeI* (Figure 1) and transferred into *E. coli* BW25113 to develop recombinant *E. coli* EG-296 strain.

The presence of the adh cassette in the transformants was verified by kanamycin resistance and PCR.[16] Transformants were grown on LB agar containing kanamycin for the selection of recombinant *E. coli* EG-296 strain. Insertion of

**Table 3.** Taguchi DOE $L_{16}$ OA

| Run | A | A*B | B | C | D | B*D | B*C | B*E | A*D | A*E | E | F | A*F | A*G | G |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **2** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| **3** | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 |
| **4** | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 |
| **5** | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 2 |
| **6** | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 1 |
| **7** | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 1 |
| **8** | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 2 |
| **9** | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| **10** | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 |
| **11** | 2 | 1 | 2 | 2 | 1 | 2 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 2 | 1 |
| **12** | 2 | 1 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 1 | 2 | 1 | 1 |
| **13** | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 2 |
| **14** | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 2 |
| **15** | 2 | 2 | 1 | 2 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 2 | 1 | 1 | 2 |
| **16** | 2 | 2 | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 1 | 2 | 2 | 1 |

the adh cassette into the genome of two antibiotic resistance transformants was verified by a PCR product of about 1300 bp (Figure 2), using genomic DNA and related primers (primers 9 and 10, Table 1).

### Biotransformation of Isobutyraldehyde to Isobutanol by *Escherichia coli* EG-296

For analyzing the isobutanol production by *E. coli* EG-296, a standard curve was drawn by GC-MS using sequential concentrations of isobutanol (Figure 3).

The Isobutanol production in the isobutyraldehyde biotransformation process was evaluated by GC-MS analysis.[17] Results showed that recombinant *E. coli* EG-296 strain could convert isobutyraldehyde into isobutanol. This is while the native *E. coli* BW25113 could not do so. The EG-296 strain produced 230 mg/L isobutanol from 600 mg/L isobutyraldehyde without any optimization.

### Optimization of Isobutanol Production

In the culture optimization for isobutanol production, the optimum values of seven factors, including carbon and nitrogen sources, inoculum age and volume, and also the culture volume were determined using the Taguchi OA design with 16 experimental trials.

The results showed that the experimental trial 6 was the best condition with about 545 mg/L isobutanol production (Table 4). The Signal-to Noise ratios vs. factor levels (Table 5) showed level 1 for glucose, glycerol, $NH_4CL$, yeast extract, inoculum age, and inoculum volume, and level 2 for culture volume as the optimized parameters.

The ANOVA analysis of the experimental data determines both individual and also interaction contributions of the factors involved in the production of isobutanol. In terms of interaction contributions, as shown in Table 6, the most important is the interaction between yeast extract and inoculum volume (16.798%). Other interaction contributions include glucose with inoculum volume (12.958%), glycerol with yeast extract (10.546%), and glucose with glycerol (6.626%). Other interaction contributions showed a negligible contribution. In addition, NH4CL (12.035%), inoculum age (10.747%), and inoculum volume (9.041%) have the highest individual contributions. Other individual factors (including glucose, glycerol, yeast extract, and medium volume) showed little contribution.

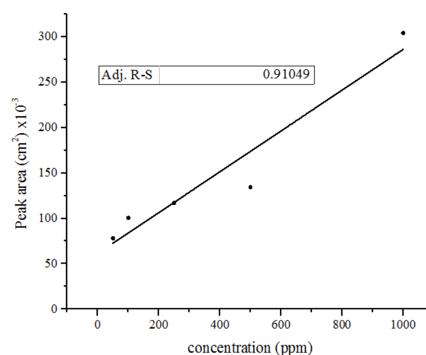


**Figure 3**. Isobutanol Standard Curve. This curve was drawn by GC-MS using sequential concentrations of isobutanol.

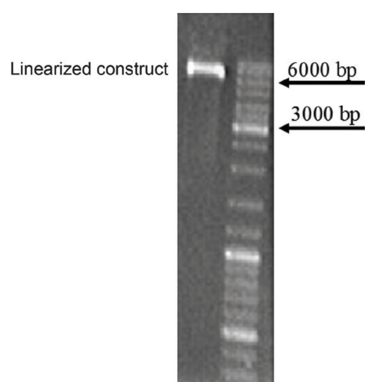


**Figure 1.** Linear pYH-185 plasmid. The final plasmid (pYH-185) contain adh cassette digested with *NdeI* enzyme. The ~7600 bp band confirmed the correct linearization.

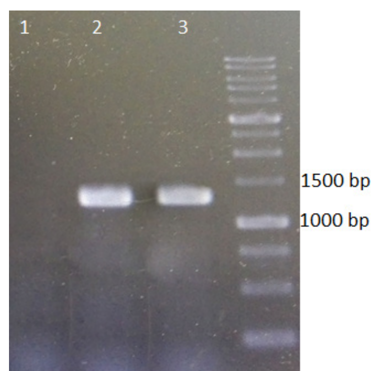


**Figure 2**. Gel Electrophoresis of PCR Product of the Integrated Cassette into *E. coli* Genome. Lane 1: negative control, lanes 2 and 3: 2 clones of recombinant *E. coli*. Presence of product with 1300 bp size indicated the proper cassette integration.

**Table 4.** Experimental Isobutanol Production Rate (mg/L)

| Run | Isobutanol (mg/L) |
|---|---|
| Trial 1 | 480.5697 |
| Trial 2 | 451.0097 |
| Trial 3 | 431.88 |
| Trial 4 | 501.38 |
| Trial 5 | 445.14 |
| Trial 6 | 545.6493 |
| Trial 7 | 268.9697 |
| Trial 8 | 528.7297 |
| Trial 9 | 399.82 |
| Trial 10 | 399.82 |
| Trial 11 | 429.94 |
| Trial 12 | 425.7897 |
| Trial 13 | 362.5897 |
| Trial 14 | 406.66 |
| Trial 15 | 468.0897 |
| Trial 16 | 382.4297 |

**Table 5.** S/N Ratio of All Factors and Their Interaction vs. Factor Levels Obtained in the Taguchi Analysis

| Factors/level | A | A*B | B | C | D | B*D | B*C | B*E | A*D | A*E | E | F | A*F | A*C | G |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 53.02 | 52.84 | 52.73 | 52.73 | 53.09 | 52.36 | 52.30 | 52.15 | 52.05 | 53.01 | 53.10 | 52.82 | 53.17 | 52.65 | 52.31 |
| 2 | 52.22 | 52.40 | 52.51 | 52.51 | 52.15 | 52.88 | 52.94 | 53.31 | 53.20 | 52.23 | 52.15 | 52.43 | 52.07 | 52.60 | 52.93 |

**Table 6.** Analysis of Variance (ANOVA) of Taguchi's OA $L_{16}$

| Factors | DOF | Sums of Squares | Variance | F-Ratio | Pure Sum | Percent |
|---|---|---|---|---|---|---|
| NH4Cl | 1 | 26,835.363 | 26,835.363 | 55.925 | 26,355.524 | 12.035 |
| INTER COLS 1 x 3 | 1 | 2,343.730 | 2,343.730 | 4.884 | 1,863.891 | 0.851 |
| Glc | 1 | 312.000 | 312.000 | 0.650 | 0.000 | 0.000 |
| Glyc | 1 | 544.142 | 544.142 | 1.134 | 64.303 | 0.029 |
| Inoc | 1 | 20,279.631 | 20,279.631 | 42.263 | 19,799.792 | 9.041 |
| INTER COLS 3 x 5 | 1 | 5,398.483 | 5,398.483 | 11.250 | 4,918.644 | 2.246 |
| INTER COLS 3 x 4 | 1 | 9,059.857 | 9,059.857 | 18.881 | 8,580.018 | 3.918 |
| INTER COLS 3 x 11 | 1 | 23,574.497 | 23,574.497 | 49.130 | 23,094.658 | 10.546 |
| INTER COLS 12 x 5 | 1 | 37,267.024 | 37,267.024 | 77.665 | 36,787.185 | 16.798 |
| INTER COLS 1 x 11 | 1 | 14,991.127 | 14,991.127 | 31.241 | 14,511.288 | 6.626 |
| Age | 1 | 24,014.649 | 24,014.649 | 50.047 | 23,534.811 | 10.747 |
| Y.E. | 1 | 2,896.257 | 2,896.257 | 6.035 | 2,416.418 | 1.103 |
| INTER COLS 1 x 12 | 1 | 28,857.185 | 28,857.185 | 60.139 | 28,377.346 | 12.958 |
| INTER COLS 1 x 15 | 1 | 798.215 | 798.215 | 1.663 | 318.376 | 0.145 |
| Vol | 1 | 6,461.215 | 6,461.215 | 13.465 | 5,981.376 | 2.731 |
| Other/Error | 32 | 15,354.845 | 479.838 | | | 10.226 |

### Effect of Carbon and Nitrogen Sources

Although carbon sources individually have no specific difference in isobutanol production, their interactions with other factors showed significant contributions. For nitrogen sources, the highest amount of isobutanol was produced in the presence of ammonium chloride as the source of nitrogen (optimal concentration of 10 g/L).

### Effect of Inoculum Age and Inoculum Volume

The results showed that although inoculum age was individually an important factor for isobutanol production, it was not the case for inoculum volume. Furthermore, the interaction of inoculum age with glycerol as a carbon source was significant (10.5%). For inoculum volume, in contrast to its individual effect, its interaction with yeast extract and glucose had the most contribution in isobutanol production. In addition, S/N ratio results showed that the optimal levels for inoculum age and inoculum volume in the fermentation process was level 1 (1%) and level 2 (5%), respectively. Finally, the optimal composition of medium for the highest production of isobutanol was 10 g/L glucose (or glycerol) as carbon source, 10 g/L $NH_4CL$ as a nitrogen source, mid-log of inoculum age, 1% inoculum volume and 25 mL of the medium amount. At optimized conditions, a confirmatory experiment was conducted using recombinant *E. coli* EG-296 strain and the produced isobutanol was measured by GS-MS analysis (Figure 4). The recombinant *E. coli* EG-296 converted isobutyraldehyde (first peak with a retention time of 1.40) into isobutanol (second peak with a retention time of 1.50). About 600 mg/L (8 mM) isobutyraldehyde was added to the optimized medium and 560 mg/L (7 mM) isobutanol was produced with 91% yield.

## Discussion

This study offers a biological approach for converting isobutyraldehyde into isobutanol as a high-value substance. Engineered *E. coli* EG-296 strain was developed by inserting *adhA* gene from *L. lactis* into the *E. coli* genome to convert isobutyraldehyde to isobutanol. In addition, the media composition was optimized by the Taguchi optimization method to increase the isobutanol production up to 91% yield.

The isobutyraldehyde is an undesirable by-product of the propylene hydroformylation process. Traditionally, the reverse reaction of this process, using catalysts such as copper and palladiumm, has been used to minimize isobutyraldehyde,[12] which is generally costly and reduces system performance. Richard Tudor and Michael Ashley used radioum as a catalyst to increase the efficiency of this process.[18] In this study, the biotransformation process was used to convert isobutyraldehyde into isobutanol.

In recent years, biotransformation has been considered to cope with environmental pollutants concerns[5,19] through various wild type and engineered microorganisms. In propylene hydroformylation, isobutyraldehyde is a pollutant for which many mechanical and chemical methods have been employed to eliminate or convert it into another compound.[6,20]

Alcohol dehydrogenase is the main enzyme present in various microorganisms that efficiently convert isobutyraldehyde into isobutanol. Many studies have been carried out to identify its highest efficiency. In a recent study by Atsumi et al[13] which compared several different alcohol-dehydrogenases, it was shown, that alcohol dehydrogenase from *L. lactis* (*ADHA*) had the highest efficiency in *E. coli*. Therefore, in the present study, this enzyme was used in an *E. coli* strain for the conversion of isobutyraldehyde into

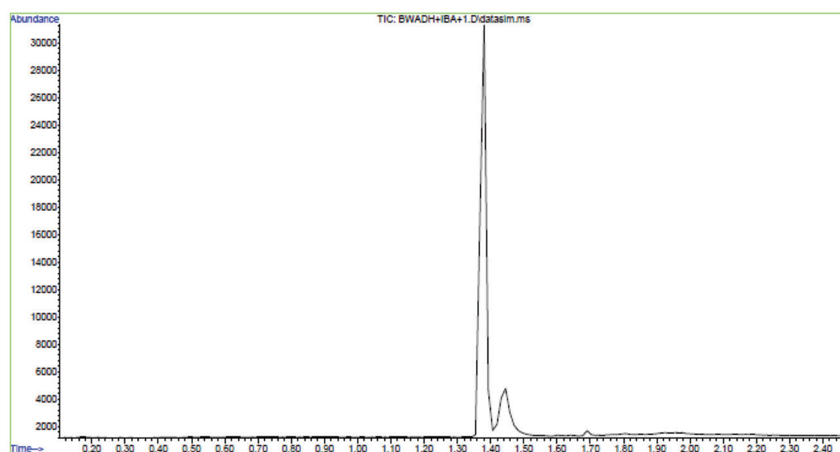


**Figure 4**. The GC-MS Analysis of Isobutyraldehyde to Isobutanol Conversion in *E. coli* EG-296 Culture. First peak with a retention time of 1.40 showed isobutyraldehyde which convert into isobutanol (second peak with a retention time of 1.50).

isobutanol. In addition, to order to increase the efficiency, some amino acids of this enzyme were changed and its codons were optimized according to the *E. coli* expression system codon preferences.[14]

Isobutanol is the most suitable substitution for fossil fuel and other biofuels such as ethanol due to many advantages, including lower hygroscopicity and corrosivity and higher energy density.[21] *The E. coli* can produce isobutanol from glucose by converting 2-ketoisovalerate (a precursor in the L-valine biosynthesis pathway) into isobutyraldehyde using a 2-keto acid decarboxylase (KDC) enzyme and then into isobutanol.[21] The strain developed in this study is a platform strain for isobutanol production. The *E. coli* EG-296 has the best alcohol dehydrogenase and therefore can be used for producing isobutanol from glucose by adding a KDC enzyme which is in progress.

In addition to Atsumi et al and Bastian et al works,[14,21] in the current study, Taguchi OA $L_{16}$ was used to investigate the impact of individual and interaction contributions of several factors, including glucose and glycerol (as carbon sources), $NH_4Cl$ and yeast extract (as nitrogen sources), inoculum age, inoculum volume, and medium volume on the production of isobutanol.

Various steps in Taguchi methodology (planning, conducting, analysis, and validation) have enabled a systematic statistical approach to optimize medium composition and increase isobutanol production in the recombinant *E. coli* EG-296 strain. The statistical evaluation indicated that the maximum individual contribution of the factors was 12.03% achieving from 10 g/L $NH_4Cl$ as the nitrogen source. In addition, the maximum interaction contribution achieved from the interaction between the inoculum volume and yeast extract was 16.79%, indicating that the nitrogen source was the most important variable in the production of isobutanol. In contrast to individual contribution of carbon sources (including glucose and glycerol), the interaction between these and other factors showed a high contribution percentage on isobutanol production. Finally, the suggested production medium for the maximum isobutanol production comprises 10 g/L glucose or glycerol as carbon source, 10 g/L$NH_4CL$ as nitrogen source, mid-log of inoculum age, %1 inoculum volume, and 25 mL medium amount. The yield of isobutanol production in optimization condition was 91%. This amount is extremely promising, however for a strain with commercial properties, further research and development must be carried out, including more genetic manipulation for removing reactions that help improve this process, investigating the production on a larger scale and further optimization of the culture medium.

## Conclusions

This work displayed a biotechnological approach for the conversion of isobutyraldehyde into isobutanol in related industries. In this way, a recombinant *E. coli* strain with a relatively optimum medium was developed to convert isobutyraldehyde into isobutanol with 91% yield. Engineered *E. coli* EG-296 strain can be used to remove isobutyraldehyde in refineries or other industries producing this chemical as a by-product. Furthermore, the strain developed in this study can be used as a platform strain for isobutanol production as a valuable biofuel, but more research and development are required for a commercial production of this product.

### Authors' Contributions

All authors contributed equally to this study.

### Conflict of Interest Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### References

1. Lin SK, Seelbach K, Wandrey C. Industrial biotransformations. Molecules. 2001;6(12):1044-6. doi:10.3390/61201044.
2. Lin B, Tao Y. Whole-cell biocatalysts by design. Microb Cell Fact. 2017;16(1):106. doi:10.1186/s12934-017-0724-7.
3. de Carvalho CCCR, da Fonseca MMR. Biotransformations. In: Reference Module in Life Sciences. Amsterdam: Elsevier; 2017.
4. Straathof AJ, Panke S, Schmid A. The production of fine chemicals

by biotransformations. Curr Opin Biotechnol. 2002;13(6):548-556. doi:10.1016/s0958-1669(02)00360-9.

5. Parales RE, Bruce NC, Schmid A, Wackett LP. Biodegradation, biotransformation, and biocatalysis (b3). Appl Environ Microbiol. 2002;68(10):4699-4709. doi:10.1128/aem.68.10.4699-4709.2002.
6. Abatenh E, Gizaw B, Tsegaye Z, Wassie M. Application of microorganisms in bioremediation-review. J Environ Microbiol. 2017;1(1):2-9.
7. Brakstad OG, Bonaunet K, Nordtug T, Johansen O. Biotransformation and dissolution of petroleum hydrocarbons in natural flowing seawater at low temperature. Biodegradation. 2004;15(5):337-346. doi:10.1023/b:biod.0000042189.69946.07.
8. Matei AV, Farcas A, Florian C, Florescu M, Coman G. Pollutants biotransformation. In: Simeonov L, Macaev F, Simeonova B, eds. Environmental Security Assessment and Management of Obsolete Pesticides in Southeast Europe. NATO Science for Peace and Security Series C: Environmental Security. Dordrecht: Springer. p. 111-117. doi:10.1007/978-94-007-6461-3_9.
9. Ojima I, Tsai CY, Tzamarioudaki M, Bonafoux D. The Hydroformylation Reaction. In: Organic Reactions. American Cancer Society; 2004. p. 1-354.
10. Ojima I, Tsai CY, Tzamarioudaki M, Bonafoux D. The Hydroformylation Reaction. Organic Reactions; 2000. doi:10.1002/0471264180.or056.01
11. Schulz HF, Bellstedt F. Hydroformylation of propene with ruthenium catalysts. Ind Eng Chem Prod Res Dev. 1973;12(3):176-83. doi:10.1021/i360047a005.
12. Falbe J, Tummes H, Hahn HD. inventors; Ruhrchemie AG, assignee. Catalytic Cleavage of Isobutyraldehyde. patent US4039584A. 1977.
13. Atsumi S, Wu TY, Eckl EM, Hawkins SD, Buelter T, Liao JC. Engineering the isobutanol biosynthetic pathway in *Escherichia coli* by comparison of three aldehyde reductase/alcohol dehydrogenase genes. Appl Microbiol Biotechnol. 2010;85(3):651-657. doi:10.1007/s00253-009-2085-6.
14. Bastian S, Liu X, Meyerowitz JT, Snow CD, Chen MM, Arnold FH. Engineered ketol-acid reductoisomerase and alcohol dehydrogenase enable anaerobic 2-methylpropan-1-ol production at theoretical yield in *Escherichia coli*. Metab Eng. 2011;13(3):345-352. doi:10.1016/j.ymben.2011.02.004.
15. Behera HT, Upadhyay AK, Raina V, Ray L. Optimization of media components for the production of N-acetylchitooligosaccharide from chitin by *Streptomyces chilikensis* through Taguchi experimental design. J Microbiol Methods. 2019;159:194-199. doi:10.1016/j.mimet.2019.03.014.
16. Hamedi Behnaq M, Karami A, Choopani A. Development of PCR-Based method for rapid detection of abrin gene. J Appl Biotechnol Rep. 2016;3(3):473-476.
17. Daryabeigi Zand A. Contribution of dissolved organic carbon in mobilizing and releasing high molecular weight polycyclic aromatic hydrocarbons from carbonaceous material-amended soil. J Appl Biotechnol Rep. 2020;7(2):125-134. doi:10.30491/jabr.2020.109288.
18. Tudor R, Ashley M. Enhancement of industrial hydroformylation processes by the adoption of rhodium-based catalyst: part I. Platin Met Rev. 2007;51(3):116-26. doi:10.1595/147106707x216855.
19. Emenike CU, Jayanthi B, Agamuthu P, Fauziah SH. Biotransformation and removal of heavy metals: a review of phytoremediation and microbial remediation assessment on contaminated soil. Environ Rev. 2018;26(2):156-168. doi:10.1139/er-2017-0045.
20. Nicolaou SA, Gaida SM, Papoutsakis ET. A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: from biofuels and chemicals, to biocatalysis and bioremediation. Metab Eng. 2010;12(4):307-331. doi:10.1016/j.ymben.2010.03.004.
21. Atsumi S, Hanai T, Liao JC. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature. 2008;451(7174):86-89. doi:10.1038/nature06450.