Biochemical Engineering Journal 133 (2018) 21–27

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Isobutanol production by a recombinant biocatalyst *Shimwellia blattae* (p424IbPSO): Study of the operational conditions

Miguel G. Acedos, Alberto Ramon, Susana de la Morena, Victoria E. Santos*, Felix Garcia-Ochoa

Chemical Engineering Department, Universidad Complutense, 28040, Madrid, Spain

ARTICLE INFO

Article history:
Received 17 October 2017
Received in revised form 29 January 2018
Accepted 2 February 2018
Available online 5 February 2018

Keywords:
Isobutanol
Glucose
Shimwellia blattae
Medium composition
Oxygen availability
pH strategy

ABSTRACT

The potential to produce high isobutanol concentrations from glucose with a new genetically modified microorganism, *Shimwellia blattae* (p424IbPSO), has been studied. The influences of several operating conditions (media composition, oxygen availability and pH) have been studied. Yeast extract concentration can be reduced 3.3 times from the more usual concentration, reducing the cost of the bioprocess, but obtaining no change in isobutanol yield: 0.173 $g_{Ib}\ g_{Gc}^{-1}$. The influence of the oxygen availability on isobutanol production has also been considered. The use of higher air proportion into sealed bottles led to the best results in isobutanol concentration and productivity: 6 g L^{-1} and 0.19 g L^{-1} h^{-1}, respectively. The study of the pH strategy shows that the best one is to begin at an initial pH value of 6.8, to let the pH fall freely to a value of 6.0 and then to control the pH at this value, obtaining about 11 g L^{-1} of isobutanol titer and about 0.286 $g_{Ib}\ g_{Gc}^{-1}$ of yield.

1. Introduction

The increase in energy consumption and the forecast about fossil fuels depletion, as well as its environmental impact, have motivated an increasing interest in the search for new alternative biofuels. Nowadays, the studies are usually focused on the quest for the best substitute of bioethanol. Heavier alcohols, of 4–6 carbon atoms, with lower hygroscopy and higher energy density are sought. Among these alcohols, isobutanol is an interesting candidate, because it is a good substitute for bioethanol due to its better properties. Isobutanol can be used as an additive for gasoline, improving its quality as fuel, or even as a replacement in the future. Moreover, isobutanol can be used as a platform chemical to produce other value-added compounds [1].

Isobutanol can be produced by native microorganisms, having been identified as a by-product in beer production [2], but nowadays the efforts are focused on the search of genetically modified microorganisms (GMO) in order to improve isobutanol production, because the low titer of isobutanol obtained from wild type strains would make the industrial bioprocess economically unfeasible [3–12]. Table 1 shows a summary of the works published testing different GMO; these works were carried out in batch operation in sealed bottles in order to avoid isobutanol evaporation. As can be seen, the highest isobutanol concentration reached was 12 g L^{-1} [13]. Nevertheless, better results can be obtained operating in fed-batch; final isobutanol concentration of 22 g L^{-1} has been reported using *Escherichia coli* JCL206 [3] in runs performed by glucose fed-batch, and around 50 g L^{-1} with the same bacteria strain by *in situ* removal of isobutanol by gas stripping [14].

The production of isobutanol with the GMO cited presents some limitations such as low isobutanol titers, inhibition of bacteria by isobutanol and cell instability. The main factor seems to be the inhibition by isobutanol [15].

A new biocatalyst, *Shimwellia blattae* modified with the plasmid p424IbPSO has been reported to produce 6 g L^{-1} on sealed bottles [7]. This GMO needs oxygen to compensate the imbalance NAD+/NADH for isobutanol production. In order to reduce the evaporation of isobutanol, the culture bottles were sealed when IPTG was added to culture media, which caused oxygen deprivation in culture bottles, stopping the growth [7]. A scheme of the metabolic pathway of this GMO is shown in Fig. 1.

The main objective of this work is to improve isobutanol production from glucose by this GMO. Accordingly, the medium composition and the values of some variables can be considered in order to improve the rate and selectivity of the bioprocess. A review of the YE concentrations employed in previous works as well as other organic nitrogen sources can be summarized in Table 1. The use of a high YE concentration in the production media for-

* Corresponding author.
E-mail address: vesantos@ucm.es (V.E. Santos).

https://doi.org/10.1016/j.bej.2018.02.002
1369-703X/

Nomenclature

A	Percentage of air volume per total volume in the vessel (%)
DCM	Dry cell mass ($g\,L^{-1}$)
C_j	Concentration of compound j ($g\,L^{-1}$)
M9	Minimal salts medium 9
ND	Not described
OD_{600}	Optical density at 600 nm
P_{Ib}	Isobutanol productivity ($g\,L^{-1}\,h^{-1}$)
$Y_{i/Gc}$	Yield of the compound i respect to the consumed glucose ($g_i\,g_{Gc}^{-1}$)
YE	Yeast extract
YNB	Yeast nitrogen base medium
YPD	Yeast extract peptone dextrose medium

Underscripts

Ac	Referred to acetic acid
Et	Referred to ethanol
Gc	Referred to the consumed glucose
i	Referred to a general compound i (i = Ac, et, ib, La, X)
Ib	Referred to isobutanol
La	Referred to lactic acid
X	Referred to biomass
0	Initial value

Superscripts

*	Referred to the maximum biomass concentration at the stationary growth phase

mulation is undesirable for the bioprocess economy at industrial scale. It is remarkable that the best results reported in these studies are achieved when YE concentration in the culture medium is about $5\,g\,L^{-1}$, although other compounds such as peptone or vitamins and amino acid solutions have been added to the medium in some cases.

As can be observed in Table 1, the initial pH and the pH strategy followed in the previous works are different from each other. Most of these works do not control the value of this variable, pH value changes freely; there is only one work performed controlling pH at shaker scale in a value of 7.0; this work reports the highest titer and yield of isobutanol [13].

The influence of oxygen has not been considered as a target for any of the works showed in Table 1. Nevertheless, oxygen availability is not the same in the different papers, although it is unknown in most of them, maybe due to the fact that the authors do not attach importance to this variable or perhaps due to the difficulty of studying it in bottles in shaker scale, as it happens with pH control.

The aim of this work is to perform a study dealing with the medium composition (trying to reduce the YE concentration), and the influence of pH and oxygen availability in the production of isobutanol with *S. blattae* (p424IbPSO), as a potential industrial isobutanol producer.

2. Materials and methods

2.1. Bacterial strain and inoculum built-up

S. blattae (p424IbPSO), is the microorganism used in this study. It has been kindly supplied by the Environmental Biotechnology Laboratory of the Biological Research Center (CSIC, Spain). The strain was stored as pellets in 1 mL Eppendorf tubes at −80 °C into 50% w/w glycerol/saline serum media prior to inoculation.

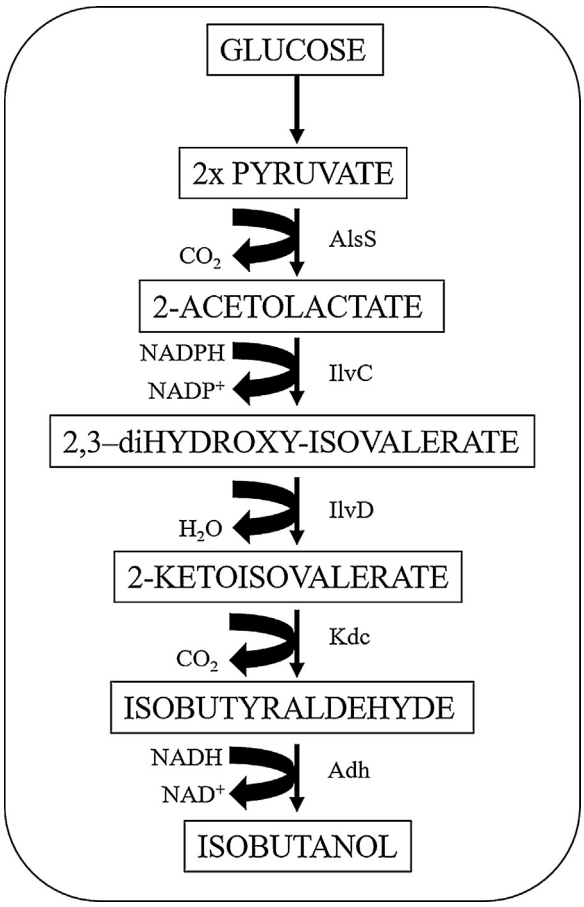

Fig. 1. Isobutanol pathway cloned in *S. blattae* (p424IbPSO). *AlsS*: acetolactate synthase; *IlvC*: acetohydroxy acid isomeroreductase; *IlvD*: dihidroxy-acid dehydratase; *Kdc*: 2-ketoacid decarboxylase; *Adh*: alcohol dehydrogenase. (Adapted from Felpeto-Santero et al. [7]).

Inoculum built-up was carried out in two stages, pre-inoculum and inoculum, in order to obtain cells in the same metabolic state and reduce experimental error, assuring experimental data reproducibility. These steps were performed in 250 mL flasks containing 50 mL of liquid media at 37 °C of temperature, in orbital shaker at 250 rpm for 12 and 3 h, respectively. M92X liquid medium containing (for a liter) 2 g NH_4Cl, 6 g KH_2PO_4, 12 g Na_2HPO_4, 1 g NaCl, 0.246 g $MgSO_4{\cdot}7H_2O$, 0.011 g $CaCl_2$ was used. This medium was supplemented with $5\,g\,L^{-1}$ YE, $20\,g\,L^{-1}$ of glucose as carbon source and $1\,mL\,L^{-1}$ of a 1000X mixture of trace elements (containing per liter: 1.5 mg nitrile-tri-acetic acid, 3 mg $MgSO_4{\cdot}7H_2O$, 0.18 mg $ZnSO_4{\cdot}7H_2O$, 0.01 mg $CuSO_4{\cdot}5H_2O$, 0.5 mg $MnSO_4{\cdot}2H_2O$, 1 mg NaCl, 0.1 mg $FeSO_4{\cdot}7H_2O$, 0.18 mg $CoSO_4{\cdot}7H_2O$, 0.3 mg $NaSeO_3{\cdot}5H_2O$, 0.02 mg $KAl(SO_4)_2{\cdot}12H_2O$, 0.01 mg H_3BO_3, 0.01 mg $Na_2MoO{\cdot}2H_2O$ and 0.025 mg $NiCl_2{\cdot}6H_2O$). The antibiotic streptomycin sulfate was used for plasmid maintenance at a concentration of $50\,mg\,L^{-1}$.

2.2. Experimental procedure

The experiments were performed both in closed bottles at an orbital shaker and a stirred tank bioreactor (STBR).

All runs (except those indicated) were performed using M92X medium, which contains (per liter) 2 g NH_4Cl, 6 g KH_2PO_4, 12 g Na_2HPO_4, 1 g NaCl, 0.246 g $MgSO_4{\cdot}7H_2O$, 0.011 g $CaCl_2$. This medium was supplemented with 1.5–5 $g\,L^{-1}$ YE (nitrogen source); $34\,g\,L^{-1}$ of glucose (carbon source) for trials at orbital shaker scale (as used by Atsumi et al.,) [16] and 50 g/L for STBR scale runs (according to Baez et al.) [14]; and $1\,mL\,L^{-1}$ of a