Full paper - 2012

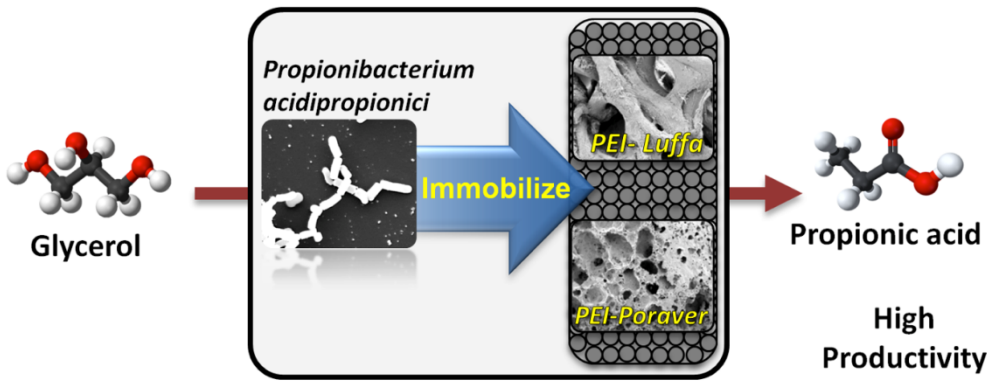
Bioresource Technology – Impact factor 4.9

Batch- and Continuous Propionic Acid Production from Glycerol Using Free and Immobilized Cells of *Propionibacterium acidipropionici*

Tarek Dishisha, Maria Teresa Alvarez and Rajni Hatti-Kaul

ABSTRACT

Propionic acid production from glycerol was studied using *Propionibacterium acidipropionici* DSM 4900 cells immobilized on polyethylenimine-treated Poraver (PEI-Poraver) and Luffa (PEI-Luffa), respectively. Using PEI-Luffa, the average productivity, yield and concentration of propionic acid from 40 g L^{-1} glycerol were 0.29 g L^{-1} h^{-1}, 0.74 mol mol^{-1} and 20.09 g L^{-1}, respectively, after four consecutive recycle-batches. PEI-Poraver supported attachment of 31 times higher amount of cells than PEI-Luffa and produced 20, 28 and 35 g L^{-1} propionic acid from 40, 65 and 85 g L^{-1} glycerol, respectively (0.61 mol_{PA} mol_{Gly}^{-1}). The corresponding production rates were 0.86, 0.43 and 0.35 g L^{-1} h^{-1}, which are the highest reported from glycerol via batch or fed-batch fermentations for equivalent propionic acid concentrations. Using a continuous mode of operation at a dilution rate of 0.1 h^{-1}, cell washout was observed in the bioreactor with free cells; however, propionic acid productivity, yield and concentration were 1.4 g L^{-1} h^{-1}, 0.86 mol_{PA} mol_{Gly}^{-1}, and 14.5 g L^{-1}, respectively, using immobilized cells in the PEI-Poraver bioreactor. The choice of the immobilization matrix can thus significantly influence the fermentation efficiency and -profile. The bioreactor using cells immobilized on PEI-Poraver allowed the fermentation of higher glycerol concentrations and provided stable and higher fermentation rates than that using free cells or the cells immobilized on PEI-Luffa.



Full paper - 2012

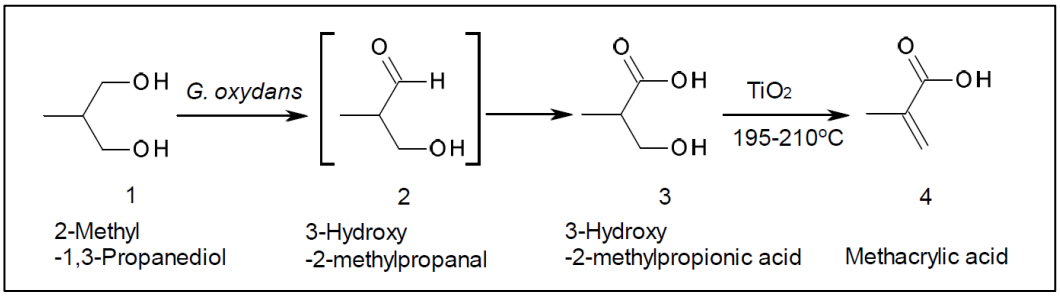
Green Chemistry – Impact factor 6.5

A New Route for the Synthesis of Methacrylic Acid from 2-Methyl-1,3-Propanediol by Integrating Biotransformation and Catalytic Dehydration

Sang-Hyun Pyo, Tarek Dishisha, Secil Dayankac, Jargalan Gerelsaikhan, Stefan Lundmark, Nicola Rehnberg, and Rajni Hatti-Kaul

ABSTRACT

Methacrylic acid was produced in high yield by an integrated process involving bioconversion of 2-methyl-1,3-propanediol (2M1,3PD) to 3-hydroxy-2-methylpropionic acid (3H2MPA) via 3-hydroxy-2-methylpropanal (3H2MPAL), and catalytic dehydration of the resulting acid. Whole cells of *Gluconobacter oxydans* grown on glycerol-based culture medium were used as the catalyst for oxidative biotransformation that involved alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes in the organism. The effect of several reaction parameters on bioconversion in a batch system was investigated to obtain 95–100% conversion of 2M1,3PD with over 95% selectivity to 3H2MPA. The optimum conditions for bioconversion were pH 6–7.5, 25–30 °C, 5–10 g substrate and 2.6 g cell (dry weight) per liter. Higher substrate concentrations led to enzyme inhibition and incomplete conversion. Loss of catalytic activity was noted during recycling of the cells. The cells were active for a longer period when used for biotransformation of 20 g per L of substrate in a continuous reactor with cell retention. The product of the bio-oxidation, 3H2MPA, was converted using titanium dioxide at 210 °C to give methacrylic acid (MA) with a yield of over 85%. The integrated process provides a new environmentally benign route for production of methacrylic acid from 2-methyl-1,3-propanediol, an industrial by-product, compared with the conventional acetone-cyanohydrin (ACH) process.



Full paper - 2013

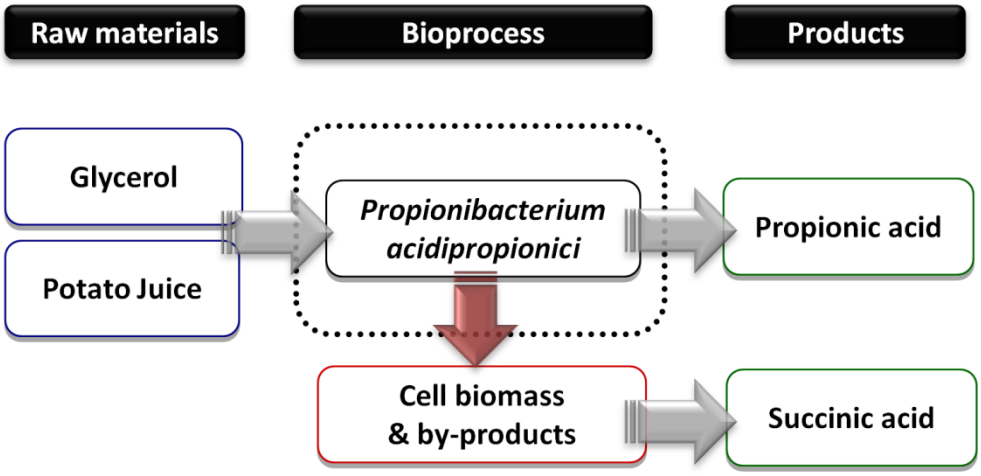
Bioresource Technology – Impact factor 4.75

An Economical Biorefinery Process for Propionic Acid Production from Glycerol and Potato Juice using High Cell Density Fermentation

Tarek Dishisha, Åke Ståhl, Stefan Lundmark and Rajni Hatti-Kaul

ABSTRACT

An economically sustainable process was developed for propionic acid production by fermentation of glycerol using *Propionibacterium acidipropionici* and potato juice, a by-product of starch processing, as a nitrogen/vitamin source. The fermentation was done as high-cell-density sequential batches with cell recycle. Propionic acid production and glycerol consumption rates were dependent on initial biomass concentration, and reached a maximum of 1.42 and 2.30 g L^{-1} h^{-1}, respectively, from 50 g L^{-1} glycerol at initial cell density of 23.7 g_{CDW} L^{-1}. Halving the concentration of nitrogen/vitamin source resulted in reduction of acetic and succinic acids yields by ~39% each. At glycerol concentrations of 85 and 120 g L^{-1}, respectively, 43.8 and 50.8 g L^{-1} propionic acid were obtained at a rate of 0.88 and 0.29 g L^{-1} h^{-1} and yield of 84 and 78 mol%. Succinic acid was 13 g% of propionic acid and could represent a potential co-product covering the cost of nitrogen/vitamin source.



Communication to the editor – 2013

Biotechnology and Bioengineering – Impact factor 3.648

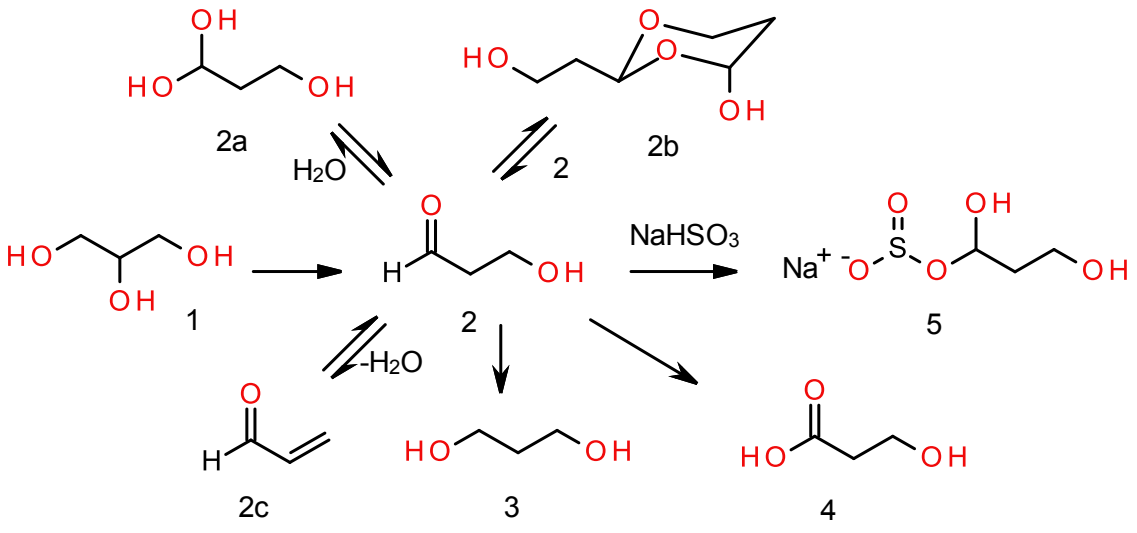
Improved Production of 3-Hydroxypropionadehyde by Complex Formation with Bisulfite during Biotransformation of Glycerol

Roya R.R. Sardari, Tarek Dishisha,* Sang-Hyun Pyo and Rajni Hatti-Kaul*

*** EQUAL CONTRIBUTION**

ABSTRACT

3-Hydroxypropionaldehyde (3HPA) is an important specialty chemical which can be produced from glycerol using resting cells of *Lactobacillus reuteri*. This biocatalytic route, however, suffers from substrate- and product-mediated loss of enzyme activity within 2 h of biotransformation. In order to overcome the inhibitory effects of 3HPA, complex formation with sodium bisulfite was investigated, optimized and applied for *in situ* capture of the aldehyde during biotransformation of glycerol in a fed-batch process. As a result, the activity of the cells was maintained for at least 18 h. The 3HPA produced per gram cell dry weight was increased 5.7 times compared to the batch production process, and 2.2 times compared to fed-batch process without *in situ* complex formation. This approach may have potential for production and *in situ* removal of 3HPA after further process development.



Full paper – 2013

Journal of Biotechnology – Impact factor 3.183

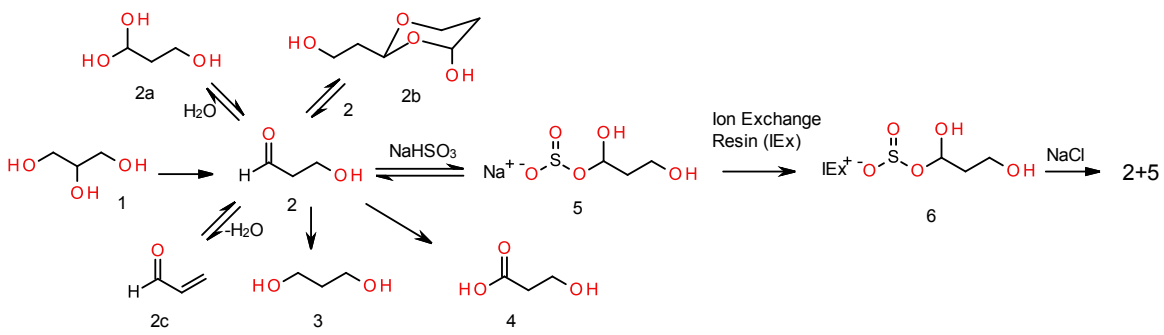
Biotransformation of glycerol to 3-hydroxypropionaldehyde: improved production by *in situ* complexation with bisulfite in a fed-batch mode and separation on anion exchanger

Roya R.R. Sardari, Tarek Dishisha*, Sang-Hyun Pyo and Rajni Hatti-Kaul*

*** EQUAL CONTRIBUTION**

ABSTRACT

3-Hydroxypropionaldehyde (3HPA) is an important C3 chemical that can be produced from renewable glycerol by resting whole cells of *Lactobacillus reuteri*. However the process efficiency is limited due to substrate inhibition, product-mediated loss of enzyme activity and cell viability, and also formation of by-products. Complex formation of 3HPA with sodium bisulfite and subsequent binding to Amberlite IRA-400 was investigated as a means of *in situ* product recovery and for overcoming inhibition. The adsorption capacity and -isotherm of the resin were evaluated using the Langmuir model. The resin exhibited maximum capacity of 2.92 mmol complex/g when equilibrated with 45 mL solution containing an equilibrium mixture of 2.74 mmol 3HPA-bisulfite complex and 2.01 mmol free 3HPA. The dynamic binding capacity based on the breakthrough curve of 3HPA and its complex on passing a solution with 2.49 mmol complex and 1.65 mmol free 3HPA was 2.01 mmol/g resin. The bound 3HPA was desorbed from the resin using 0.20 M NaCl with a high purity as a mixture of complexed- and free 3HPA at a ratio of 0.77 mol/mol. Fed-batch biotransformation of glycerol (818.85 mmol) with *in situ* 3HPA complexation and separation on the bisulfite-functionalized resin resulted in an improved process with consumption of 481.36 mmol glycerol yielding 325.54 mmol 3HPA at a rate of 17.13 mmol/h and a yield of 68 mol%. Also, the cell activity was maintained for at least 28 h.



Full Paper - 2014

For Microbial Cell Factories – Impact factor 4.25

Flux Analysis of the *Lactobacillus reuteri* Propanediol-Utilization Pathway for Production of 3-Hydroxypropionaldehyde, 3-Hydroxypropionic Acid and 1,3-Propanediol from Glycerol

Tarek Dishisha, Luciana P. Pereyra, Sang-Hyun Pyo, Robert A. Britton and Rajni Hatti-Kaul*

BACKGROUND

Lactobacillus reuteri can convert glycerol to 3-hydroxypropionic acid (3HP) and 1,3-propanediol (1,3PDO) with 3-hydroxypropionaldehyde (3HPA) as an intermediate thanks to the enzymes and structural proteins encoded in its propanediol-utilization (*pdu*) operon. Since 3HP, 1,3PDO and 3HPA are important building blocks for the bio-based chemical industry, *L. reuteri* can be an attractive candidate for their industrial production. However, little is known about the kinetics of glycerol utilization in the Pdu pathway in *L. reuteri*. In this study, the metabolic fluxes through the Pdu pathway were determined as a first step towards optimizing the production of 3HPA and co-production of 3HP and 1,3PDO from glycerol by *L. reuteri*. Whole resting cells of wild-type (DSM 20016) and recombinant (RBRB3007, with overexpressed *pdu* operon) strains were used as biocatalysts.

RESULTS

The rate of conversion of glycerol to 3HPA by the resting cells of *L. reuteri* was evaluated by in situ complexation of the aldehyde with carbohydrazide in order to avoid its toxicity to the cells. Under operational conditions, the 3HPA specific production rate of the RPRB3007 strain was 1.9 times higher than that of the wild-type strain (1718.2 and 889.0 $mg/g_{CDW}.h$, respectively). Metabolic flux analysis of glycerol to 1,3PDO and 3HP in the cells using controlled variable-volume fed-batch operation showed that the maximum specific production rates of 3HP and 1,3PDO were 110.8 and 93.7 $mg/g_{CDW}.h$, respectively, for the wild-type strain and 179.2 and 151.4 $mg/g_{CDW}.h$, respectively, for the RPRB3007 strain. The cumulative molar yield of the two compounds was 1 mol/mol glycerol and their molar ratio was ~1 $mol_{3HP}/mol_{1,3PDO}$. A balance of redox equivalents between the glycerol oxidative and reductive pathway branches led to equimolar amounts of the two products.

CONCLUSIONS

Improved specific production rates for 3HPA, 3HP and 1,3PDO were obtained with resting cells of the engineered RBRB3007 strain, which highlights the potential of metabolic engineering to render an industrially sound strain. The use of resting cells for the production of 1,3PDO and 3HP is promising for the industrial-scale production of these compounds. This is the first report on the production of 3HP and 1,3PDO as sole products using the wild-type *L. reuteri* or the RPRB3007 mutant strain.

Full Paper - 2014

For Journal of Biotechnology (Impact factor 2.89)

Semicarbazide-Functionalized Resin as a New Scavenger for *in situ* Recovery of 3-Hydroxypropionaldehyde during Biotransformation of Glycerol by *Lactobacillus reuteri*

Roya R. R. Sardari, Tarek Dishisha, Sang-Hyun Pyo and Rajni Hatti-Kaul*

ABSTRACT

3-Hydroxypropionaldehyde (3HPA), a potential C3-platform chemical for a biobased industry, is produced from glycerol using *Lactobacillus reuteri* through its glycerol dehydratase activity. However, the process is characterized by low yield and -productivity due to toxic effects of 3HPA on the biocatalyst activity. In this study, a semicarbazide-functionalized resin was prepared, evaluated for adsorption and *in situ* recovery of 3HPA during biotransformation of glycerol. Adsorption of 3HPA onto the resin was characterized as "*S-curve* model", increasing with increasing initial 3HPA concentration, and reached a maximum of 9.48 mmol/g_{resin} at 71.54 mM 3HPA used. Desorption of 3HPA was evaluated using water and different acids, and was enhanced by acetic acid with organic modifiers. Repeated adsorption-desorption of 3HPA in batch resulted in elution of 13-66.5% of the bound 3HPA during at least three sequential cycles using water and acetic acid, respectively as eluants. Using the resin for *in situ* product removal led to more than 2 times higher productivity of 3HPA.

Full paper – 2014

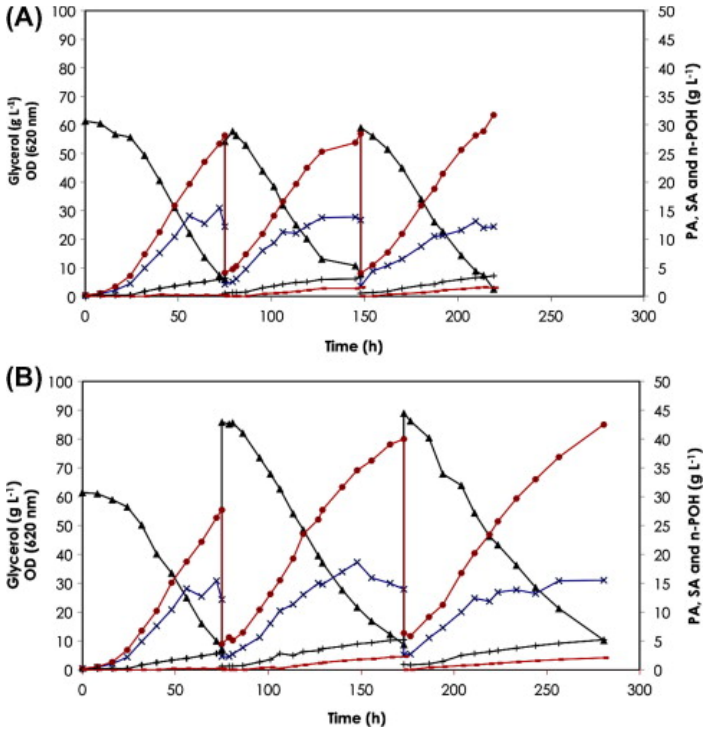
For Bioresource Technology – Impact factor 5.039

Improved Propionic Acid Production from Glycerol: Combining Cyclic Batch and Sequential Batch Fermentations with Optimal Nutrient Composition

*Tarek Dishisha, Mohammad HA Ibrahim, Victor Hugo Cavero, Maria Teresa Alvarez and Rajni Hatti-Kaul**

ABSTRACT

Propionic acid was produced from glycerol using *Propionibacterium acidipropionici*. In this study, the impact of the concentrations of carbon and nitrogen sources, and of different modes of high cell density fermentations on process kinetics and -efficiency was investigated. Three-way ANOVA analysis and batch cultivations at varying C/N ratios at pH 6.5 revealed that propionic acid production rate is significantly influenced by yeast extract concentration. Glycerol to yeast extract ratio (ww^{-1}) of 3:1 was required for complete glycerol consumption, while maintaining the volumetric productivity. Using this optimum C/N ratio for propionic acid production in cyclic batch fermentation gave propionate yield upto 93 mol% and productivity of 0.53 $gL^{-1}h^{-1}$. Moreover, sequential batch fermentation with cell recycling resulted in production rates exceeding 1 $gL^{-1}h^{-1}$ at initial glycerol upto 120 gL^{-1}, and a maximum of 1.63 $gL^{-1}h^{-1}$ from 90 gL^{-1} glycerol.



Full Paper 2015

For Microbial Cell Factories – 4.22

Bio-Based 3-Hydroxypropionic- and Acrylic Acid Production from Biodiesel-Derived Glycerol via Integrated Microbial and Chemical Catalysis

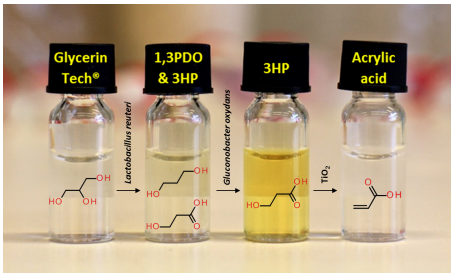
*Tarek Dishisha, Sang-Hyun Pyo and Rajni Hatti-Kaul**

ABSTRACT

Background: 3-Hydroxypropionic acid (3HP) and acrylic acid (AA) are industrially important platform- and secondary chemical, respectively. Their production from renewable resources by environment-friendly processes is desirable. In the present study, both chemicals were almost quantitatively produced from biodiesel-derived glycerol by an integrated process involving microbial and chemical catalysis.

Results: Glycerol was initially converted in a fed-batch mode of operation to equimolar quantities of 3HP and 1,3-propanediol (1,3PDO) under anaerobic conditions using resting cells of *Lactobacillus reuteri* as a biocatalyst. The feeding rate of glycerol was controlled at 62.5 mg/g_{CDW}.h which is half the maximum metabolic flux of glycerol to 3HP and 1,3PDO through the *L. reuteri* propanediol-utilization (*pdu*) pathway to prevent accumulation of the inhibitory intermediate, 3-hydroxypronionaldehyde (3HPA). Subsequently, the cell-free supernatant containing the mixture of 3HP and 1,3PDO was subjected to selective oxidation under aerobic conditions using resting cells of *Gluconobacter oxydans* where 1,3PDO was quantitatively converted to 3HP in a batch system. The optimum conditions for the bioconversion were 10 g/L substrate and 5.2 g/L cell dry weight. Higher substrate concentrations led to enzyme inhibition and incomplete conversion. The resulting solution of 3HP was dehydrated to AA over titanium dioxide (TiO_2) at 230 °C with a yield of >95%.

Conclusions: The present study represents the first report on an integrated process for production of acrylic acid at high purity and -yield from glycerol through 3HP as intermediate without any purification step. The proposed process could have potential for industrial production of 3HP and AA after further optimization.



Research Paper 2016

For Journal of Biotechnology – Impact Factor 2.871

Selective oxidation of trimethylolpropane to 2,2-bis(hydroxymethy)butyric acid using growing cells of *Corynebacterium sp.* ATCC 21245

Mahmoud Sayed, Tarek Dishisha, Waiel F. Sayed, Wesam M.A. Salem, Hanan M. Temerk, and Sang-Hyun Pyo

ABSTRACT

Multifunctional chemicals including hydroxycarboxylic acids are gaining increasing interest due to their growing applications in the polymer industry. One approach for their production is a biological selective oxidation of polyols, which is difficult to achieve by conventional chemical catalysis. In the present study, trimethylolpropane (TMP), a trihydric alcohol, was subjected to selective oxidation using growing cells of *Corynebacterium sp.* ATCC 21245 as a biocatalyst and yielding the dihydroxy-monocarboxylic acid, 2,2-bis(hydroxymethyl)butyric acid (BHMB). The study revealed that co-substrates are crucial for this reaction. Among the different evaluated co-substrates, a mixture of glucose, xylose and acetate at a ratio of 5:5:2 was found optimum. The environmental conditions for optimal biotransformation in a bioreactor were pH 8, 1 v/v/m airflow and 500 rpm stirring speed. Under these conditions in batch mode of operation, 70.6% of 5 g/l TMP was converted to BHMB in 10 days. For recovery of the product the adsorption pattern of BHMB to the anion exchange resin, Ambersep® 900 (OH^-), was investigated in batch and column experiments giving maximum static and dynamic binding capacities of 135 and 144 mg/g resin, respectively. The resin was successfully applied for recovery of BHMB from the fermentation broth.