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A new route for the synthesis of methacrylic acid from 2-methyl-1,3-propanediol by integrating biotransformation and catalytic dehydration

Sang-Hyun Pyo^{a,*}, Tarek Dishisha^a, Secil Dayankac^a, Jargalan Gerelsaikhan^a,
Stefan Lundmark^b, Nicola Rehnberg^c, and Rajni Hatti-Kaul^a

^a Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, Box 124, SE-221 00 Lund, Sweden

^b Perstorp AB, 284 80 Perstorp, Sweden

^c Strategic R&D, Bona AB, Box 210 74, 200 21 Malmö, Sweden

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Paper

A new route for the synthesis of methacrylic acid from 2-methyl-1,3-propanediol by integrating biotransformation and catalytic dehydration†

Sang-Hyun Pyo^a, Tarek Dishisha^a, Secil Dayanak^a, Jargalan Gerelsaikhan^a, Stefan Lundmark^b, Nicola Rehnberg^c, and Rajni Hatti-Kaul^a

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Methacrylic acid was produced at 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 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/L 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.

Introduction

Methacrylic acid (MA) and methyl methacrylate (MMA) are important monomers for a range of polymer products; the major product is poly(methyl methacrylate) (PMMA), with an annual global consumption of 2.1 million tonnes.¹ Approximately 80% of the global production of MMA is achieved using the well known acetone-cyanohydrin (ACH) process, in which acetone and hydrogen cyanide are reacted with excess concentrated sulphuric acid to produce the methacrylamide acid sulphate², followed by treating with excess aqueous methanol, and hydrolysis and esterification of the amide to yield a mixture of MMA and MA. However, there are serious problems with this route due to the use of highly toxic raw materials, high process cost, and formation of significant amounts of by-products, which have to be disposed.^{1,2}

The ACH process is estimated to result in emission of 5.5 kg CO₂/kg MMA, which is much higher than that from other platform chemicals such as propylene (1.1 kg CO₂/kg), ethylene (1.1 kg CO₂/kg), and vinyl chloride monomer (1.5 kg CO₂/kg).³ Therefore, among several platform chemicals, a greener production route of MMA can be expected to be highly beneficial for the environment. Different alternative processes have been proposed since the 1980s to replace the ACH technology. Some of them are close to commercialization, while others, although attractive from environmental and economic points of view, are still in the research stage.²

An earlier study has reported the production of an analogous compound, acrylic acid from 3-hydroxypropionic acid in a continuous process with >98% conversion and >99% selectivity using titanium oxide as the catalyst at 180 °C.⁴ Also, dehydration of lactic acid to obtain acrylic acid has been studied,^{5,6} but the product yield was too low for commercial application. A similar process for the production of methacrylic acid would require 3-hydroxy-2-methyl propionic acid (3H2MPA, isohydroxybutyric acid) as the substrate. Synthesis of this chemical has been achieved by enantioselective oxidation of 2-methyl-1,3-propanediol (2M1,3PD) using an acetic acid bacterium, *Acetobacter pasteurianus*.⁷ 2M1,3PD is a by-product of the butanediol production process involving hydroformylation reaction of allyl alcohol and CO/H₂ gas mixture in the presence of a catalyst, and hence is a cheap and commercially available substrate.⁷⁻⁹ Recently, the possibility of 2M1,3PD production

^a Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, Box 124, SE-221 00 Lund, Sweden. E-mail: Sang-Hyun.Pyo@biotek.lu.se; Fax: +46-46-222-4713; Tel: +46-46-222-4838

^b Perstorp AB, 284 80 Perstorp, Sweden

^c Strategic R&D, Bona AB, Box 210 74, 200 21 Malmö, Sweden

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from renewable resources has been suggested by fermentation of glycerol and thin stillage obtained from biofuel production process, by *Citrobacter freundii*.¹⁰

In the present report, a process comprising oxidation of 2M1,3PD to 3H2MPA via 3-hydroxy-2-methylpropanal (3H2PAL), followed by catalytic dehydration is proposed as a new synthetic route to produce methacrylic acid. *Gluconobacter oxydans* was selected as the microorganism for oxidation of 2M1,3PD to 3H2MPA, which was subsequently dehydrated to methacrylic acid using TiO₂ (Scheme 1). The latter has been used as an environmentally friendly photocatalyst in water treatment and organic synthesis, and is known to be a non-toxic and biocompatible material.^{11,12}

Scheme 1. Conversion of 2-methyl-1,3-propanediol to methacrylic acid via 3-hydroxy-2-methylpropionic acid by microbial conversion and catalytic dehydration.

Materials and Methods

2.1. Materials

2-Methyl-1,3-propanediol was a product of Perstorp AB (Sweden). Methacrylic acid, 2,4-dinitrophenylhydrazine (99%, DNPH), CDCl₃, DMSO-d₆, CaCO₃, and glycerol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium phosphate salts, acetonitrile, ethyl acetate and dichloromethane were purchased from MERCK (Germany), while TrizmaTM was from VWR international, and yeast extract (BactoTM) was obtained from BDH (NJ, USA). *G. oxydans* (DSM 50049) was purchased from DSMZ (Deutsche Sammlung von Mikroorganismen, Germany).

2.2. Maintenance and cultivation of *G. oxydans*

G. oxydans was maintained on solid medium containing per liter: 50 g glucose, 10 g yeast extract, 20 g CaCO₃, and 15 g agar-agar at pH 6.8. The agar slants were inoculated with the stock culture of the microorganism in 20 % (v/v) glycerol, and incubated at 28 °C for 2 days, and then stored at 4 °C. To prepare the preculture, cells from the surface of the slants were transferred to 100 mL fermentation medium in 1 L baffled Erlenmeyer flask, containing (per liter): 10 g glycerol, 10 g yeast extract, 5 g KH₂PO₄ at pH 5.5. The flasks were incubated in an orbital shaker incubator (New Brunswick, Innova 4430, Edison, USA) at 28 °C and 200 rpm for 4 days. The pre-culture (75 mL, OD₆₂₀ = 2.8) was used to inoculate 1.5 L fermentation medium in a 3 L bioreactor (Applikon, Microbial Biobundle, The Netherlands). The fermentation was monitored and controlled through ez-control unit. The temperature was controlled at 28 °C and pH at 5.5 through addition of 5 N NH₄OH and 5 N HCl. Stirrer speed was 500 rpm and a constant airflow at 1 vvm was maintained during the whole fermentation. Sterile polypropylene glycol in water (1:1) was used as antifoam and was added when required. The fermentation lasted for 2 days and samples were collected and analysed for optical density (OD), cell dry weight (CDW) and concentration of glycerol, acetic acid, succinic acid, and dihydroxyacetone (DHA) using HPLC. After 48 hours, the

fermentation was terminated and the cell broth was harvested and stored at 4 °C until use.

2.3. Biotransformation of 2-methyl-1,3-propanediol to 2-methyl-3-hydroxypropionic acid

G. oxydans cells were obtained by centrifugation of the culture broth at 10 000 × g for 2 min and followed by washing with 0.1 M Tris-HCl buffer (pH 7.2) to remove the residual culture media. Oxidation of 2M1,3PD (2.5-30 g/L) using *G. oxydans* cells was studied in 1 mL reaction volume in 5 mL vials placed in a thermomixer (MKR 13, HLC Biotech, Germany) at 500 rpm. The reaction conditions were optimized with respect to reaction time, substrate- and cell concentration at 28 °C and pH 7.2.

Fig. 1. Bioreactor system used for continuous biotransformation of 2-methyl-1,3-propanediol to 3-hydroxy-2-methylpropionic acid using resting cells of *G. oxydans*. The cells were retained by a tangential flow microfiltration module (3) integrated with the bioreactor (1). Pump (2) was used to feed the substrate 2M1,3PD to (1) and remove the cell-free product from the membrane filter at a rate of 0.5 mL/min, while pump (4) was used to recirculate the reaction suspension with the cells between the reactor and the microfiltration unit at a rate of 30 mL/min.

Continuous biotransformation of 2M1,3PD was performed using the system illustrated in Fig. 1, composed of a 3 L bioreactor connected to a tangential flow microfilter, (Pellicon XL, 0.45 µm, 50 cm² filtration area, Millipore, Bedford, MA). Two pumps were used – one for re-circulating the reaction suspension between the bioreactor and the filtration system at a rate of 30 mL/min, and the other for feeding the fresh substrate solution and removing the cell free product stream at a constant rate. During the whole run, the stirrer speed was kept constant at 1000 rpm, air flow at 1 vvm, pH at 7.2 though addition of 5 N NH₄OH, and temperature at 28 °C. The cells used for the biotransformation were previously grown as described above, recovered from the fermentation broth by centrifugation at 15 000 × g for 10 min. The reaction was performed in 1 L working volume containing 5 g 2M1,3PD and 5.85 g cells (dry weight) in 0.05 M Tris-HCl (pH 7.2), initially in a batch mode for 7.2 h followed by a continuous mode by feeding 20 g/L 2M1,3PD in the same buffer at a dilution rate of 0.03 h⁻¹, removing the cell-free product as a permeate from the microfiltration unit while recycling the retained cells, for a period of 107 hours.

The biotransformation experiments were performed in two independent runs and analyses of the reaction components was performed in duplicates.

2.4. Catalytic dehydration of 3-hydroxy-2-methylpropionic acid to methacrylic acid

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3H2MPA, obtained as a product of batch biotransformation using *G. oxydans* cells, was used without further treatment for catalytic dehydration. A simple flow reaction cell was designed using a stainless tube (4.5 x 200 mm) packed with 3 g TiO₂ and placed in an oven. Twenty five millilitres of a pre-heated 3H2MPA solution (5 g/L) was fed to the pre-heated reaction tube using a quantitative pump (Pump 2248, Pharmacia LKB, Sweden) at different flow rates for providing different residence times. The reaction temperature was varied in the range of 175-210 °C. The eluate containing MA was condensed in a cold water bath and collected.

2.5. Analyses and structure elucidation

Growth of *G. oxydans* cells was monitored by measuring optical density (OD) at 620 nm (UV-Vis Spectrophotometer, Ultrospec 1000, Pharmacia Biotech, Sweden). For determination of cell dry weight, cells were recovered from 10 mL broth by centrifugation (10 000 × g for 2 min), and washed using distilled water and dried overnight in an oven at 110 °C.

The concentrations of glycerol and dihydroxyacetone (DHA) were determined by HPLC (Dionex, Ultimate RS 3000 system, USA) equipped with UV/VIS (RS-3000 RI-101 Shodex), and an oven (RS 3000). Separation of the compounds was done on an Aminex HPX 87-H ion exclusion chromatographic column (300 mm long, ID 7.8 mm, particle diameter 9 µm, Bio-Rad Laboratories, CA, USA). The column temperature was maintained at 40 °C. Samples from the bioreactor were diluted with MilliQ quality water. Thirty microliter sample was injected into the column and eluted using water:acetonitrile (65:35) containing 0.5 mM sulfuric acid as the mobile phase at a flow rate of 0.5 mL/min, and the eluted products were detected at 270 nm. For determination of acetic acid and succinic acid, the same chromatographic column was used, and peak separation was done using 5 mM sulphuric acid at a flow rate of 0.6 mL/min. Oven temperature was kept at 55 °C and detection was done using RI detector (ERC-inc, Kawaguchi, Japan).

Quantitative analyses of reaction components were performed using gas chromatography (GC, Varian 430-GC, Varian, USA) equipped with FactorFour Capillary column, VF-1ms (Varian, 15M × 0.25mm) and a flame ionization detector. The initial column oven temperature was increased from 50 to 250 °C at a rate of 20 °C/min. The samples, diluted with acetonitrile to a concentration of 0.1-1 g/L, were injected in split injection mode of 10 % at 275 °C. The percent conversion of 2M1,3PD and formation of the products were calculated from the peak areas on the gas chromatograms (Supplementary data). Commercially available 2M1,3PD and MA were used as standards, while 3H2MPA was prepared from reaction of 2M1,3PD using sufficient amount of cells to reach 100 % conversion. All samples were analysed in duplicates and data provided is an average of the replicates. The molecular masses of all substances were measured by GC-MS (Varian 431-GC, Varian 210-MS) equipped with FactorFour Capillary column, VF-5ms (Varian, 30M × 0.25 mm). The initial column oven temperature was increased from 50 °C to 275 °C at a rate of 15 °C/min. The diluted samples were injected at 275 °C.

The kinetic parameters for the oxidation of 2M1,3PD and 3H2MPAL by ADH and ALDH, respectively, were determined from the Lineweaver–Burk plot of the initial reaction velocity vs. the substrate concentration:

$$1/v = (K_m/V_{\max})/[S] + 1/V_{\max} \quad (\text{Eq. 1})$$

where [S], $V_{\max}$, and K_m are substrate concentration, maximum reaction rate and Michaelis constant, respectively.

The identity of 3H2MPAL was confirmed by reactive extraction with DNPH, which is a general method for confirming aldehydes.¹³ To the reaction solution recovered after centrifugation, was added 0.1 M DNPH solution followed by extraction with ethyl acetate. The organic phase was dried and subjected to ¹³C-NMR analysis (Supplementary data).

3H2MPA was isolated from the reaction mixture by simple filtration followed by water evaporation under reduced pressure. The structure of 3H2MPA was elucidated by ¹H and ¹³C-NMR using 400 MHz NMR (Bruker, UltraShield Plus 400, Germany), (Supplementary data).

3. Results and discussion

3.1. Cultivation of *G. oxydans* cells

Acetic acid bacteria are characterised by their ability to catalyse incomplete oxidation of alcohols and sugars to the corresponding aldehydes, ketones and organic acids. This was translated into several industrial processes for production of valuable chemicals such as dihydroxyacetone (DHA) from glycerol, L-sorbose and L-sorbose from D-sorbitol, and D-gluconic acid, 2-keto- and 2,5-diketo- gluconic acid (precursor of vitamin C) from glucose using *Gluconobacter oxydans* and acetic acid from ethanol using *Acetobacter* spp.¹⁴ In this study, *G. oxydans* DSM 50049 cells obtained by cultivation in a glycerol-based culture medium were used to catalyse the conversion of 2M1,3PD to 3H2MPA. The growth curve of *G. oxydans* showed no lag phase and the microorganism grew logarithmically at maximum specific growth rate ($\mu_{\max}$ of 0.213 h⁻¹) and doubling time (τ = 3.25 h) during the first 12 hours of cultivation (Fig. 2).

Fig. 2. Growth curve of *G. oxydans* showing Ln OD_{620 nm} for calculation of growth rate (x), % dissolved oxygen / 10 (●), and concentrations of glycerol (▲), DHA (■), acetic acid (*), and succinic acid (○). The values are the mean of two independent runs, and measurements performed in duplicates.

This was accompanied by increase in oxygen uptake rate as shown by the decrease in the dissolved oxygen (%DO) from 90 to 5% of saturation. Complete consumption of glycerol and production of 9.3 g/L DHA was observed after 18 hours of cultivation. Subsequently, the microorganism assimilated the produced DHA via the pentose phosphate pathway¹⁵ and the growth rate was decreased to 0.04 h⁻¹ (τ = 17.3 h). During this period the %DO was stabilized around 20% and then increased

Table 1. Results of biotransformation of varying concentrations of 2-methyl-1,3-propanediol by 2.5 mg dry weight/mL *G. oxydans* cells at 29 °C and pH 7.2 during initial 3 h

Run	Substrate (2M1,3PD)			Product		ADH reaction	ALDH reaction	
	Initial		Remained	3H2MPAL (M)	3H2MPA (M)	Conversion ^a (%)	Substrate ^b (M)	Conversion ^c (%)
	g/L	M	2M1,3PD (M)					
1	5	0.055	0.017	0.000	0.038	68.9	0.038	100.0
2	7.5	0.083	0.031	0.005	0.046	62.3	0.052	89.6
3	10	0.111	0.053	0.009	0.049	52.5	0.058	84.3
4	12.5	0.139	0.071	0.014	0.054	48.8	0.068	79.8
5	15	0.166	0.098	0.019	0.050	41.3	0.069	72.9
6	20	0.222	0.163	0.027	0.032	26.6	0.059	54.9
7	30	0.333	0.278	0.045	0.010	16.5	0.055	17.6
V_{max} (μmol/min/mg)						0.265		0.213
K_m (M)						0.122		0.059

Abbreviations: 2M1,3PD: 2-methyl-1,3-propanediol, 3H2MPAL: 3-hydroxy-2-methylpropanal, 3H2MPA: 3-hydroxy-2-methylpropionic acid.

^aConversion of 2M1,3PD. ^bSubstrate calculated as sum of 3H2MPAL and 3H2MPA used in ALDH reaction.

^cConversion to 3H2MPA

gradually to 100% at the end of day 2. The final cell density reached was 1.3 g cell dry weight per liter. These cells were collected for use in the biotransformation of 2M1,3PD.

3.2. Bioconversion of 2-methyl-1,3-propanediol by *G. oxydans*

3.2.1. Characterization of bioconversion and reusability of cells

Oxidative biotransformation of 2M1,3PD using resting cells of *G. oxydans* was investigated under conditions considered optimal (25–30 °C and pH 6–8) for the organism.^{7,16,17} The reaction was studied in 1 mL volume at varying substrate concentration (5, 7.5, 10, 12.5, 15, 20 and 30 g/L), pH 7.2 (0.1 M Tris-HCl), 29 °C using 2.6 mg of cell dry weight/mL. Substrate conversion, reaction rate and product formation were followed and are summarised in Table 1. Complete conversion of 5 g/L 2M1,3PD to 3H2MPA was achieved in 9–12 h (not shown), while 98% of 7.5 g/L was converted to 3H2MPA in 24 hours (Fig. 3).

Higher substrate concentrations, 10–12.5 and 15 g/L resulted in 89% and 80.5% conversion, respectively, in 24 h. 3H2MPA and its methyl ester, known as Roche ester, can be used as major building blocks in organic synthesis.¹⁸

Molinari et al.^{8,16} have earlier reported the oxidative conversion of several aliphatic alcohols such as 1-propanol, 1-butanol, 2-methyl-1-butanol, and 2M1,3PD to the corresponding carboxylic acid mainly by *Acetobacter* spp. Also enantioselective oxidation of (R,S)-2-phenyl-1-propanol to (S)-2-phenylpropanoic acid was achieved at 45–50% molar conversion in 24 h by *G. oxydans*.¹⁷ Two membrane-bound enzymes in *G. oxydans*, ADH and ALDH are suggested to be involved in the conversion of the diols to the hydroxyacid. The evidence for the involvement of the membrane-bound quinoxinoprotein ADH in *G. oxydans* DSM 2003 for biooxidation of 1,2-propanediol to lactaldehyde, was recently obtained in a study using ADH deficient mutant.¹⁹ This study also proved the contribution of several ALDHs towards the subsequent oxidation of lactaldehyde to D-(-)-lactic acid.

Fig. 3. Profiles of reaction substrate (◆, 2-methyl-1,3-propanediol), intermediate (■, 3-hydroxy-2-methylpropanal), and product (▲, 3-hydroxy-2-methylpropionic acid) with time during biotransformation using 2.6 mg cells (dry weight) *G. oxydans* at pH 7.2 (0.1M Tris-HCl), in 1 mL reaction volume in 5 mL vial at 29 °C. The reaction was carried out using 7.5 g/L substrate. The values are the mean of two independent runs, and measurements performed in duplicates.

Fig. 3 shows that the reaction with 7.5 g/L substrate reached over 60% conversion within the first 3 hours; subsequently the reaction rate decreased with time and the substrate transformation was marginal after 8 h. The aldehyde intermediate was totally consumed and the yield of the final product was 98%. The initial reaction rates for ADH and ALDH with different substrate concentrations were calculated based on the reactions for 3 h (Table 1).

As seen in Fig. 4A, the rate for ADH catalysed reaction increased with increase in 2M1,3PD concentration up to 0.2 M and then decreased suggesting substrate inhibition. Product inhibition by the aldehyde intermediate 3H2MPAL could be ignored since it was continuously converted to 3H2MPA by ALDH reaction, and hence its concentration was kept sufficiently low (below 0.05 M). The amount of substrate for ALDH catalysed reaction was calculated as sum of residual 3H2MPAL and the product 3H2MPA (Table 1), which showed that the reaction rate was faster than that of ADH reaction at the prevailing substrate concentrations (Fig. 4A).

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Kinetic parameters were determined from the Lineweaver–Burk plot (Fig.4B); K_m (M) and V_{max} ($\mu\text{mol}/\text{min}/\text{mg}$) values for ADH were 0.122 and 0.265, and for ALDH were 0.059 and 0.213, respectively (Table 1). It seems that the inhibition for the ALDH reaction occurs at lower substrate concentration than that for the ADH reaction. On the other hand, product inhibition was not apparent since 3H2MPA could not be produced at sufficiently high concentration due to the inhibitory effect of the substrate.

Fig. 4. (A) Initial reaction rates of ADH (◆) and ALDH (■) calculated from the data collected for 3 h during biotransformation with *G. oxydans* cells with different substrate concentrations in 0.1M Tris-HCl buffer, pH 7.2 at 28 °C. (B) Lineweaver–Burk plot of ADH (◆) ($y = 0.4614x + 3.7809$, $R^2 = 0.9888$) and ALDH reaction (■) ($y = 0.2809x + 4.6978$, $R^2 = 0.9559$) using the same data.

Fig. 5. Plots of initial reaction rates versus substrate concentration during biotransformation of 2-methyl-1,3-propanediol with varying cell concentration in 0.1M Tris-HCl buffer, pH 7.2 at 28 °C. The symbols indicate cell dry weight of and 2.5 g/L (◆), 5 g/L (■) and 10 g/L (▲).

Different concentrations of 2M1,3PD were treated with varying cell concentrations (2.5–10 mg/mL). The plot of reaction

rate versus substrate concentration indicates decrease in specific reaction rate with increase in cell concentration, although showing similar trend of substrate inhibition above 0.2 mM 2M1,3PD at each cell concentration used (Fig. 5).

Recycling of the cells for the biotransformation of 2M1,3PD was investigated. As seen in Fig. 6, while the substrate conversion was efficient during the first run, the activity of the cells during the second run was decreased resulting in a mixture of 28% unconsumed substrate, 33% aldehyde intermediate and 39% acid product. In the subsequent run, only 30% conversion of substrate took place to give 4% acid and 26% aldehyde, suggesting faster inactivation of ALDH.

Fig. 6. Reusability of *G. oxydans* cells for selective oxidation of 2-methyl-1,3-propanediol. Five milligram of 2-methyl-1,3-propanediol was reacted using 2.6 mg cells at pH 7.2 (0.1M Tris-HCl) in 1 mL reaction volume in 5 mL vial at 28 °C. The symbols indicate the proportions of 2-methyl-1,3-propanediol (▲), 3-hydroxy-2-methyl-propanal (■), and 3-hydroxy-2-methylpropionic acid (□). After each run, the cells were recovered by centrifugation and used for the subsequent run.

The loss of activity of the cells is most likely partly due to the limitation of dissolved oxygen during transfer from one batch to the other; acetic acid bacteria are known to be highly sensitive to oxygen limitation²⁰. Moreover, the accumulation of the aldehyde intermediate as seen in Fig. 6 would also lead to enzyme inhibition.

3.2.2. Biotransformation in continuous cultivation with cell retention

The oxidative biotransformation of 2M1,3PD was then studied using the *G. oxydans* cells in a bioreactor integrated with membrane filtration to enable cell retention and recycle (Fig. 7). Initially the reaction was started in a batch mode using 5 g/L 2M1,3PD, which gave 90% conversion in 7.2 h. The reaction was then shifted to a continuous mode at residence time of 33.3 h using a feed of 20 g/L substrate. The concentrations of both substrate and product increased gradually in the outlet stream.

The 3H2MPA concentration in the outlet reached a maximum of 8.5 g/L after 66.8 hours from the start of the feeding and subsequently the concentration was decreased down to 5.4 g/L when the reaction was terminated. The substrate concentration continued increasing gradually and reached 13.7 g/L at the end of the experiment. The dissolved oxygen was constant during the entire process. Compared to results obtained above with the recycled cells (25 h), the cells in the continuous mode were maintained with higher catalytic activity for a longer period of time (~120 h) and left no residual aldehyde intermediate during the entire reaction time, even though the substrate concentration used was at an inhibitory level for the activity of the cells. This

could be due to more favorable conditions such as oxygen supply and pH control maintained by feeding NH_4OH . Comparison between different processes used industrially or experimentally for acetic acid production from ethanol by *Acetobacter* spp. showed that industrial bioreactors such as Frings acetator, submerged and semi-continuous are characterized by higher final acid concentration but limited volumetric productivity, while in the high-cell-density bioreactors with cell-recycle, the volumetric productivity was almost 10 times higher despite the 2-3 times lower final acid concentration obtained.²⁰

Fig. 7. Time course of the biotransformation of 2-methyl-1,3-propanediol (◆) to 3-hydroxyl-2-methylpropionic acid (▲) via 3-hydroxyl-2-methylpropanal (■) using 5.85 g cell dry weight/L in batch and continuous modes. Initial batch mode reaction using 5 g/L substrate for 7.2 h was followed by the reaction in continuous mode using feeding solution of 20 g/L substrate at a dilution rate of 0.03 h^{-1} , 28 °C, 1000 rpm, and 1 vvm.

3.3. Production of methacrylic acid by dehydration of 3-hydroxy-2-methylpropionic acid

3H2MPA was subjected to dehydration by passing through a bed of TiO_2 . Ti is the ninth most abundant element on Earth; more than 5.28 millions tons being produced in 2008.^{12,21} TiO_2 shows low toxicity and has been approved as a food colorant (E-171 in EU legislation).²² Over 95% conversion of 3H2MPA was achieved with over 85% yield of MA as observed by GC analysis (Fig 8, Supplementary data). The reaction temperature and residence time in the reactor were critical parameters to reach high conversion. At flow rate of 0.1 mL/min, the conversion was slightly increased from 5.7 to 16.3 % with increase in temperature from 175 °C to 195 °C, but a drastic increase in the conversion to near 100% was achieved at 210 °C. Meanwhile, at 0.025 mL/min, the degree of conversion was increased at a relatively low temperature, reaching 88% at 195 °C and 100% at 210 °C (Fig. 8). Flow rates faster than 0.1 mL/min at 210 °C gave lower conversion (Fig. 8, Insert). In comparison, dehydration of 3-hydroxypropionic acid was possible at 180 °C.⁴

It seems that 3H2MPA with higher boiling point, 252.9 °C at 760 mmHg (118-120 °C at 2 mmHg) required higher reaction temperature for dehydration. Nevertheless, the conversion and selectivity showed much better results compared to dehydration of lactic acid,^{4,6} which has been investigated with increasing interest, during past couple of decades, as one possible route for the bio-based production of acrylic acid.²³

Fig. 8. Effects of reaction temperature and feeding rate on dehydration of 3-hydroxy-2-methylpropionic acid. The reactions were carried out using 25 mL of 5 g/L substrate. The symbols represent feeding rate at 0.025 mL/min (□) and 0.1 mL/min (■). Insert shows the effect of feeding rate at 210 °C.

However, dehydration of secondary hydroxyl group has proven to be unexpectedly difficult, which is quite resistant toward hydrolysis.²³ β -Hydroxy acid was shown to be more easily dehydrated into acrylic acid than α -hydroxyl of lactic acid.⁴ There is however no report about the dehydration of α -substituted β -hydroxy acid to prepare methacrylic acid.

Conclusions

This study provides an alternative greener route for the synthesis of methacrylic acid from 2-methyl-1,3-propanediol, an inexpensive industrial by-product.⁷⁻⁹ All reactions were performed in aqueous media without using organic solvents, and separation and/or pre-treatment were not required due to high product yield in each step. The process provides significant environmental advantages over the acetone-cyanohydrin process used for methacrylic production, which is based on fossil gas or oil as raw material, uses corrosive sulfuric acid and toxic HCN, and produces 1.5 tons of ammonium bisulfate as waste per ton of MMA.²⁴ Hence, it provides a good model for comparing life cycle assessment of the new and the traditional routes. The approach is applicable for the selective oxidation of other polyols. Further investigations are however required to develop the system to allow effective conversion of higher concentrations of substrate such as in the process for the production of acetic acid from ethanol using *Acetobacter* sp.²⁰

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Notes and references

- ^a Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, Box 124, SE-221 00 Lund, Sweden. E-mail: Sang-Hyun.Pyo@biotek.lu.se; Fax: +46-46-222-4713; Tel: +46-46-222-4838
- ^b Perstorp AB, 284 80 Perstorp, Sweden
- ^c Strategic R&D, Bona AB, Box 210 74, 200 21 Malmö, Sweden
- † Electronic Supplementary Information (ESI) available: [S1. Gas chromatograms. S2. GC-MS and NMR data]. See DOI: 10.1039/b000000x/

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