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Biotechnological Production of Acrylic Acid from Biomass

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ABSTRACT

The quantitative conversion of lactic acid to acrylic acid would open a new market for renewable resources within the chemical industry. This paper focuses on the theoretical ways of producing acrylics out of renewable resources. It summarizes possible fermentation routes from carbohydrates to acrylic acid and reviews former research activities in this area. It also illustrates novel approaches involving recombinant microorganisms.

Index Entries: *Clostridium propionicum*; acrylic acid; lactic acid; biomass.

INTRODUCTION

Acrylic acid (CH_2=CH—COOH) is a commodity chemical of considerable value (1.65 ECU/kg) *(1)*. Acrylic acid and its amide and ester derivatives are principle materials in the manufacture of polymeric products. Numerous applications in surface coatings, textiles, adhesives, paper treatment, polishes, leather, fibers, detergents, and super-absorbent materials such as diapers are known *(2)*. The worldwide production of compounds associated with acrylics is estimated to be approx 2.8 million tons/yr. Currently 100% of acrylic acid is produced out of fossil oil, most of it via direct oxidation of propene *(3)*.

This project focuses on the biotechnological production of acrylic acid from organic feed stocks. A few microorganisms have been described to produce acrylic acid as a biochemical intermediate substance *(4–6)*, but observations of free acrylic acid in biological systems are rare. Anerobic formation of acrylic acid is found in the direct reduction pathway of lactic acid (CH_3—CH_2OH—COOH). This conversion is a dehydration reaction. The enzyme responsible for this conversion, lactyl-CoA dehydratase, has

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been partially purified from *Clostridium propionicum (7)*. When this microorganism uses lactic acid as the energy source, the main metabolic products are propionic acid (2/3) and acetic acid (1/3).

According to Sanseverino *(8)* and Akedo *(9)*, the propionic pathway may be blocked with 3-butynoic acid. Nevertheless, acrylate concentrations never exceeded 1% of the initial substrate concentrations. These low yields are due to the enrichment of reduction equivalents like ferredoxin, rubredoxin, and flavodoxin, which inhibit further growth of cells. These reduction equivalents should be regenerated if the cells were provided with an electron acceptor. Within this study, 3-butynoic acid with and without methylenblue as an electron acceptor was investigated.

Possible Strategies for the Production of Acrylic Acid

Acrylic acid normally is not a metabolic end-product. In principle, the following strategies may be followed to obtain high acrylic acid concentrations:

A. Biotechnological production of lactic acid out of biomass, product concentration, purification, and finally chemical conversion of lactic acid to acrylic acid (*see* pathways 1 and 5 in Fig. 1). Unfortunately, this conversion gives quite low yields because of decarbonylation, decarboxylation, and condensation reactions, which mainly leads to acetaldehyde or 2,3-pentanedione *(10)*.
B. Conversion of complex substrates to lactic acid via conventional homofermentative lactic acid fermentation and further conversion of the lactic acid fermentation broth with *Clostridium propionicum* to acrylic acid (pathways 1 and 4 in Fig. 1). This microorganism has been demonstrated to convert 3 mol lactic acid into 1 mol acetate and 2 mol propionate via the acrylyl-CoA pathway. Normally, acrylate is only produced after blocking the direct-reduction pathway. Two PhD studies dealing with the influence of 3-butynoic acid as blocker are available already *(8,9)*. In both works, acrylic acid concentrations never exceeded 1% of the initial substrate concentration.
C. Insertion of lactyl-CoA dehydratase gene into lactic acid bacteria or into *Clostridium butyricum* (pathways 1 and 4 in Fig. 1).
D. Direct conversion of complex substrates into propionic acid with cocultures of *Lactobacilli* and for example, *Propionibacterium shermanii* with further conversion of propionate to acrylate by *Clostridium propionicum* in the presence of an electron acceptor. This approach using methylene blue is described by O'Brien et al. *(1)*. They observed conversion rates of propionate into acrylate up to 18.5% by resting cells (pathways 2 and 6 in Fig. 1).

The proposed strategy 2, above from lactate (or L-alanine) to acrylic acid with *Clostridium propionicum* is shown in Fig. 2.

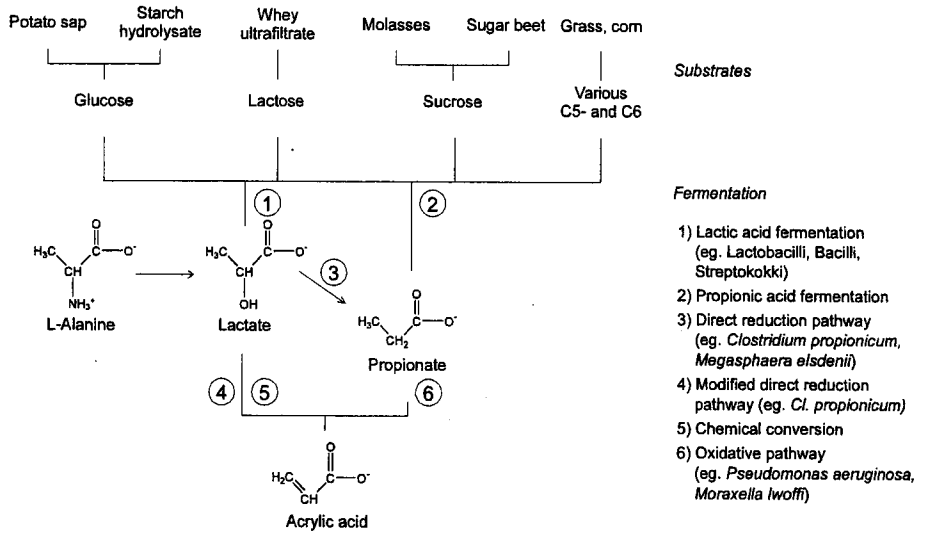

Fig. 1. Possible fermentation routes from carbohydrates to acrylic acid. *Clostridium propionicum* is normally producing propionate from lactate via the direct reduction pathway *(3)*. Modifying this pathway enables the production of acrylic acid from lactic acid *(5)*. Further details of this modification, *see* Fig. 2.

Within this project, the investigations will focus on biotechnological routes to obtain acrylic acid.

MATERIAL AND METHODS

Clostridium propionicum DSM 1682 was obtained from the German Strain Collection. Inoculum preparation and batch experiments were done on standard medium: 1000 mL bidistilled water contain L-alanine (3.0 g), peptone (3.0 g), yeast extract (4.0 g), cysteine-hydrochloride (0.3 g), $MgSO_4 \cdot 7\ H_2O$ (0.1 g), $FeSO_4 \cdot 7\ H_2O$ (0.018 g), 1 *M* K-PO_4-Puffer (pH 7.1) (5.0 mL), $CaSO_4$ (saturated solution, 2.5 mL), Resazurin (1 mg). Experiments with lactic acid as carbon source contained 3 mL 90% lactic acid solution instead of L-alanine. 3-butynoic acid was synthesized according to Heilbron et al. *(12)* using petrolether instead of recommended ether. Experiments were done strictly anerobic under nitrogen atmosphere in 500-mL flasks.

Analysis of lactose, glucose, galactose, ethanol, acrylate, butyrate, acetate, propionate, and lactic acid were done with HPLC (HP 1100C) using Bio-Rad (Hercules, CA) HPX-87H column and RI (HP1047 A) detector. The mobile phase was 0.01 *N* H_2SO_4 (flow 0.45 mL/min, temperature 55°C). Samples were diluted 1:5 with 0.01 *N* H_2SO_4 and centrifugated (Beckmann, Fullerton, CA, GS-15, 10 min). 5 μL of the supernatant were injected into the HPLC.

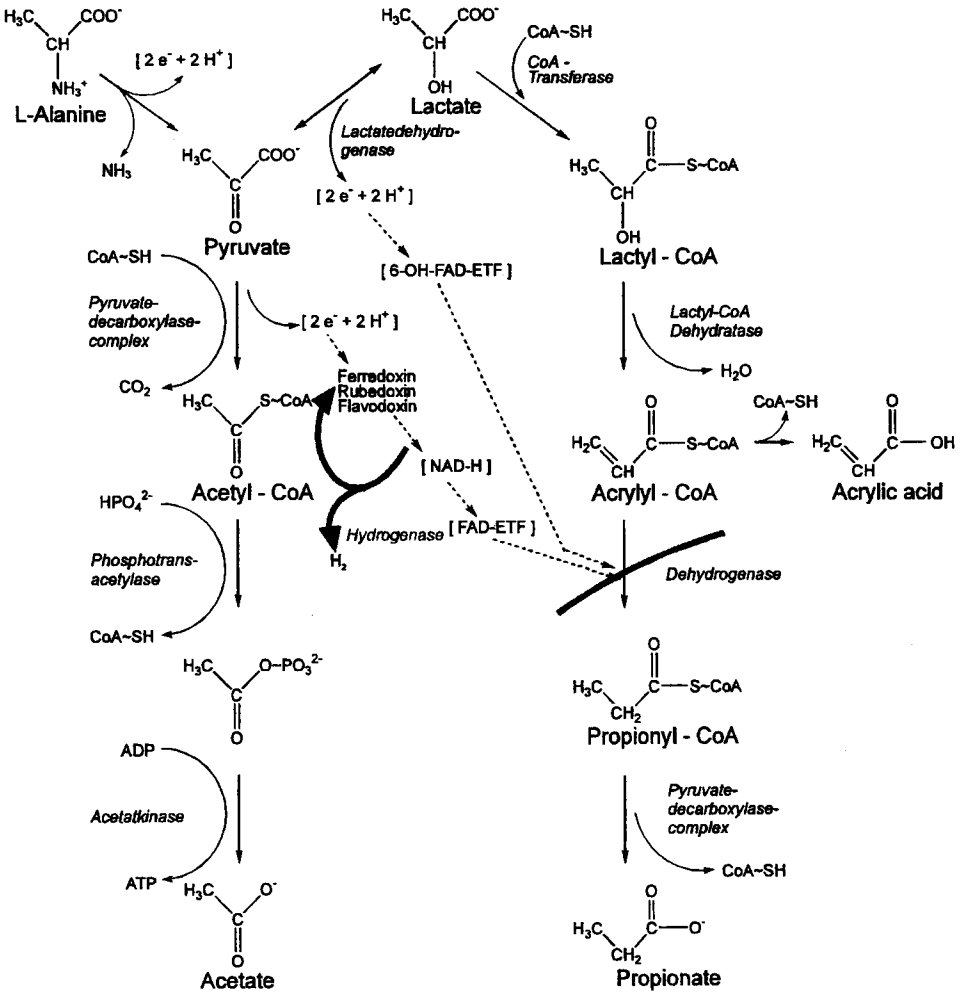

Fig. 2. Direct reduction pathway of *Clostridium propionicum* (pathway 3 of Fig. 1). Blocking the dehydrogenase and inserting a hydrogenase for regeneration of reduction equivalents (e.g., ferredoxin) should lead the microorganism to produce mainly acrylic acid (pathway 4 in Fig. 1). L-alanine undergoes oxidative deamination to pyruvate. A part of the pyruvate is oxidized to acetate and another part of pyruvate is reduced to propionate, balancing the reduction equivalents that are derived from L-alanine deamination and pyruvate oxidation *(9)*.

Protein was detected using Bio-Rad Micro Assay in microtiter plates after base hydrolysis of cell protein.

RESULTS AND DISCUSSIONS

Growth of *Clostridium propionicum* on L-alanine

As indicated in Fig. 3, growth of *C. propionicum* took place within less than 20 h after inoculation. Highest productivities were observed after

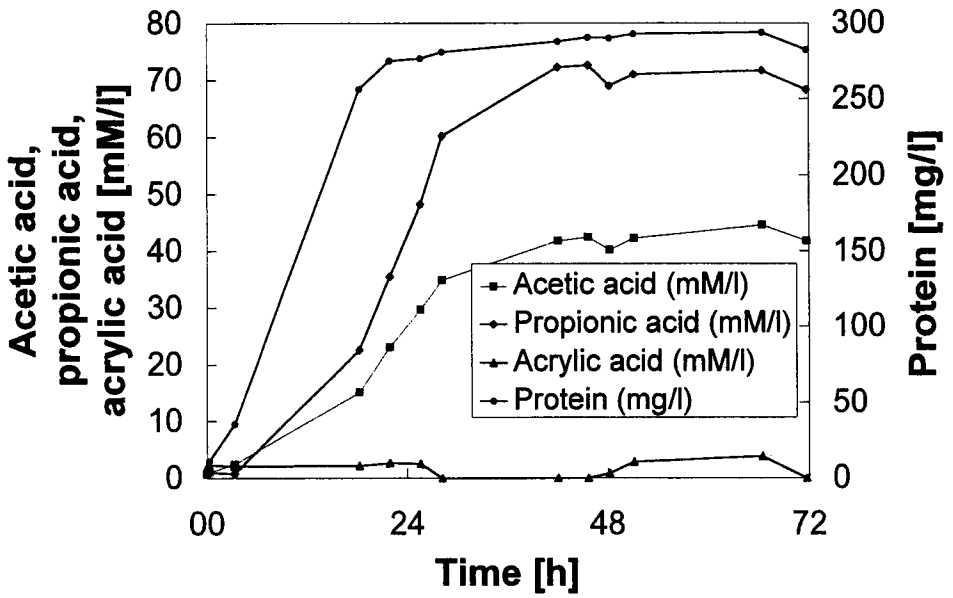

Fig. 3. Growth of *Clostridium propionicum* in batch with L-alanine as the main carbon source.

maximum growth. The ratio of propionic acid to acetic acid is between 1.5–2:1. This confirms the suggested metabolism of Fig. 2, according to which the ratio should be 2:1. Almost no acrylic acid could be detected.

Growth of *Clostridium Propionicum* on Lactic Acid

The conversion of renewable resources to acrylic acid is only feasible when cheap and easily available substrates can be used. The production of lactic acid out of various sugars and crop hydrolysates have been demonstrated frequently and may be realized at costs, which are compareable with production costs out of fossil oil. From the economical point of view, biotechnological production of acrylic acid has to be based on the conversion of lactic acid and not on alanine.

Figure 4 demonstrates that *C. propionicum* may be grown successfully on lactic acid. Preculturing of the inocula on lactic acid is necessary to obtain sufficient conversion rates and propionic and acetic acid yields. The ratio of propionate to acetate is again approx 1.5:1. Almost no formation of acrylate was observed.

3-Butynoic Acid as Blocker of Propionate Pathway

In order to achieve an accumulation of acrylic acid, the desired metabolic intermediate, a classical approach is to inhibit a certain reaction or enzyme activity with use of substrate analogs that might be metabolic inhibitors. 3-Butynoic acid ($HC{\equiv}C{-}CH_2{-}COOH$) has a structure similar

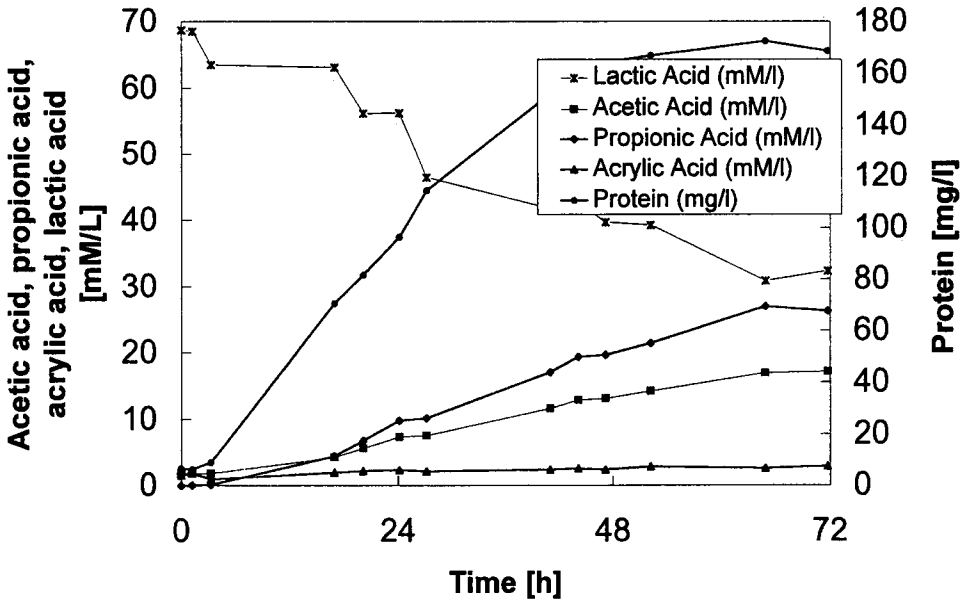

Fig. 4. Growth of *Clostridium propionicum* in batch with lactic acid as the main carbon source.

to acrylic acid. The hypothesis proposed is that this analog may inhibit the activity of propionyl-CoA dehydrogenase so that acrylyl-CoA is not further metabolized and acrylate would accumulate *(9)*.

Figure 5 and Table 1 show the influence of 3-butynoic acid on the growth and product formation of *C. propionicum*. Different amounts of 3-butynoic acid were added after 20 h fermentation time. The formation of acetate is not influenced at all by 3-butynoic acid. The increase in protein and in propionate is significantly lower when butynoic acid is added. Increasing the concentration of 3-butynoic acid decreases the propionate formation. This indicates that 3-butynoic acid is a suitable blocker of propionyl-CoA dehydrogenase. However, no additional acrylate was observed. This is caused by the formation and enrichment of reduction equivalents as NADH and 6-OH-FAD-ETF. Only if a regeneration mechanism for these reduction equivalents is provided, acrylic acid will be accumulated.

Methylene Blue as Electron Acceptor

In alanine fermentation, methylene blue can be used as an alternative electron acceptor. This concept is supported by an observation that *C. propionicum* cells can oxidize propionate to acrylate anaerobically in the presence of methylene blue as an electron acceptor replacing oxygen *(7)*. Although methylene blue is known as a bacteriostatic agent, no inhibition at methylene blue concentrations of 0.1 and 0.3% (wt/vol) for enterococci and lactic acid streptococci was observed *(13)*. Whenever we added meth-

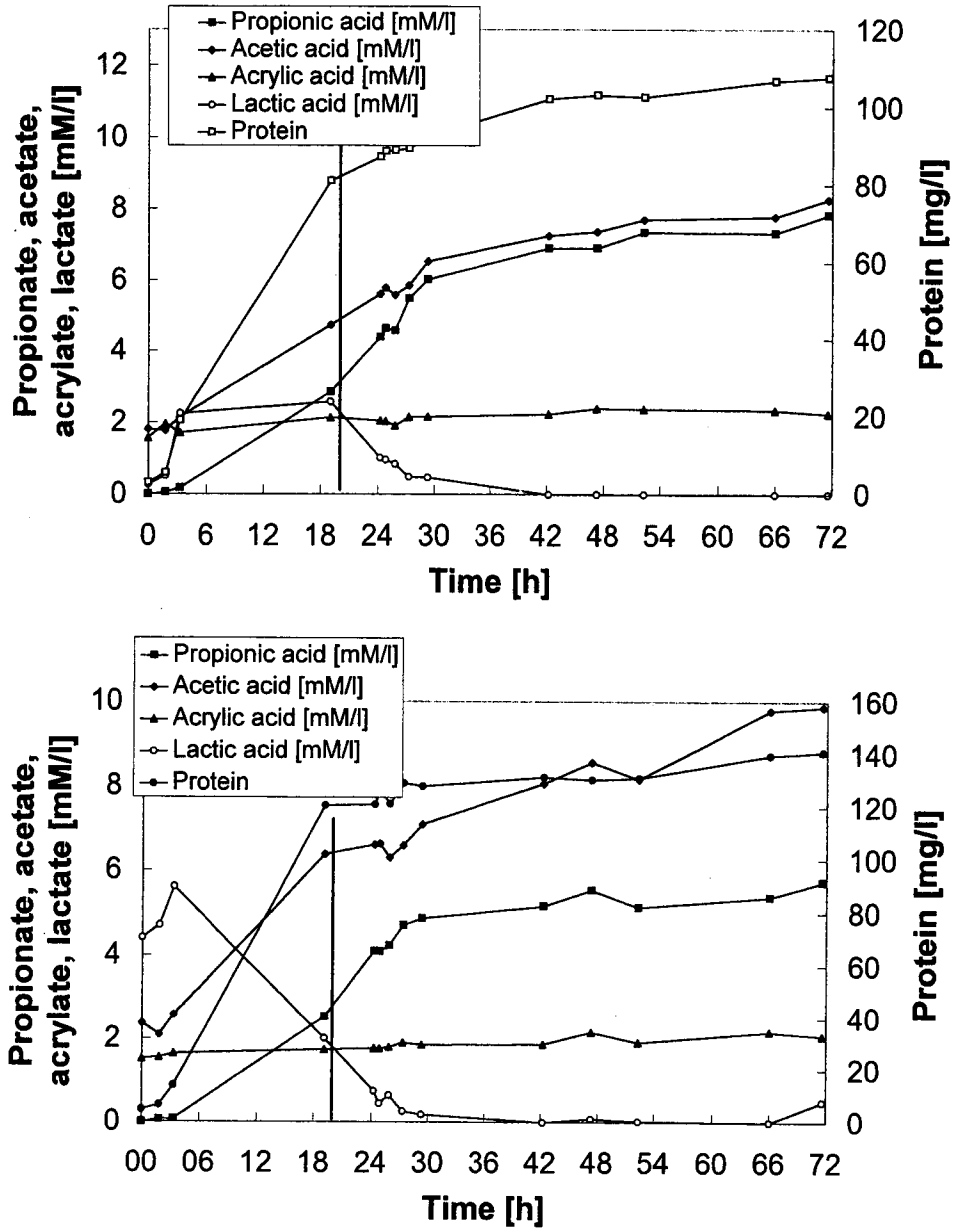

Fig. 5. Growth of *C. propionicum* on alanine. 3-Butynoic acid was added after 20 h fermentation, graph on the top with 2.5 m*M* 3 butynoic acid, graph below with 10 m*M* butynoic acid. Both graphs represent the average values of two replicate experiments.

Table 1
Influence of 3-Butynoic Acid on Growth of *C. propionicum*

	Concentration of 3-Butynoic acid				
	0 m*M*	2.5 m*M*	5 m*M*	7.5 m*M*	10 m*M*
Protein (mg/L)	46.3	26.5	27.4	20.3	22.6
Acetate (m*M*/L)	0.26	0.21	0.19	0.21	0.07
Propionate (m*M*/L)	0.44	0.37	0.25	0.24	0.12
Acrylate (m*M*/L)	0.05	0.01	0.02	0.02	0.02

Protein, acetate, propionate, and acrylate indicates the amount of protein or acid formed after addition of 3-butynoic acid after 20 h fermentation time

ylene blue to a final concentration of 0.05 to 0.2% (w/v) prior to inoculation or after 20 h fermentation, no further growth or conversion of substrate could be observed.

CONCLUSION

The major problems of acrylate formation from lactic acid and from renewable resources are the regeneration of reduction equivalents (ferredoxin, NADH) and the sufficient inhibition of propionyl-CoA dehydrogenase. It was demonstrated, that 3-butynoic acid is a suitable inhibitor of propionyl-CoA dehydrogenase, although required concentrations of this blocker are quite high (>10 m*M*).

It also was demonstrated, that the regeneration of reduction equivalents with methylene blue as the electron acceptor is not an ideal one. Further experiments especially the insertion of a hydrogenase gene into *C. propionicum* are necessary and in progress.

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