Substitution of yeast extract in industrially relevant bioprocesses (SubBioPro)

Anja Kuenz[1], Susan Krull[1], Victoria Hancock[1], Silvia Brock[1], Ulf Prüße[1]

- **Complex nitrogen sources (e.g. yeast extract) are a significant cost factor in industrial bioprocesses. The aim is to reduce costs through the hydrolysis of agricultural raw materials.**
- **After optimizing the chemical hydrolysis of protein-rich agricultural raw materials, the cost of the nitrogen source could be reduced to around 10%.**
- **In the biotechnological production of L-lactic acid and 1,3-propanediol, yeast and meat extract were completely or largely replaced, without any loss in product concentration or maximum productivity.**

Background and Objectives

Due to the scarcity of fossil resources biotechnological processes, in which renewable raw materials can be converted into industrially important raw materials, are gaining in importance. In many biotechnological processes yeast extract, as a complex nutrient source of amino acids, nucleotides, trace elements and vitamins, is required for the cell growth of microorganisms. However, yeast extract represents a high cost factor in industrial bioprocesses. Therefore, the use of inexpensive, protein-rich agricultural raw materials as a source of nutrients is desirable. However, their usage usually results in lower product concentrations and productivities in the fermentation process, so that this project focused on:

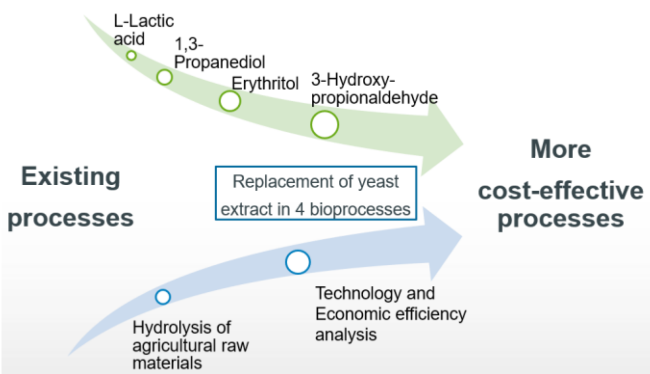
- Optimization of the chemical hydrolysis of the protein-rich agricultural raw materials.
- Elucidation of the nutritional requirements for the fermentation processes of L-lactic acid, erythritol, 1,3-propanediol and biomass cultivation for the production of 3-hydroxypropionaldehyde.
- Replacement of the complex source of nutrients with hydrolysed protein-rich agricultural raw materials.
- Technological and economic efficiency analysis of hydrolysis and bioprocesses.

Procedure

To optimize the hydrolysis of the agricultural raw materials, i.e. rapeseed meal and ProtiGrain® (DDGS), digestions were carried out between 110 and 160 °C and between 1 and 24 hours. In addition, the molarity of the used sulfuric acid was varied between 0.1 and 3M with the aim of generating as many free amino acids as possible. In parallel, the nutritional requirements of the microorganisms were examined. Then, the complex nitrogen sources were replaced by the hydrolysates of the agricultural raw materials and, if necessary, small amounts of vitamins and amino acids were added.

Results

The optimization of the hydrolysis of protein-rich agricultural raw materials shows that the molarity of sulfuric acid has a significant influence. The amount of free amino nitrogen increases with increasing molarity of the acid. In order to achieve acceptable amino nitrogen concentrations with reduced sulfuric acid molarity, the temperature was raised to 160 °C. This increase of 50 °C achieved 81.3% of the amino nitrogen concentration with 1M sulfuric acid compared to using 3M sulfuric acid. As a result of this optimization, the costs for the hydrolysate rapeseed meal are 8.4-14%, i.e. around 10% of the original costs of the yeast extract with the same nitrogen content.



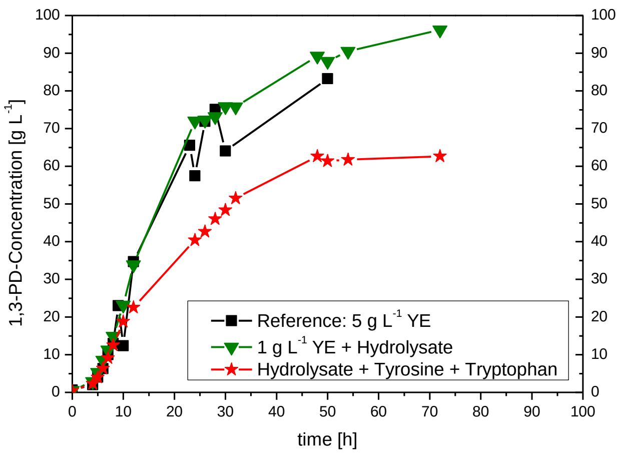
Source: Thünen-Institute/Anja Kuenz (2021).

In the biotechnological production of L-lactic acid, erythritol, 1,3-propanediol and 3-hydroxypropionaldehyde, the nutritional requirements (amino acids, nucleotides, trace elements and vitamins) of the used microorganisms were partially clarified.

On this basis, the complex nitrogen sources (yeast extract, meat extract, peptone) were successfully replaced with the rapeseed meal hydrolysate for two of the four examined bioprocesses, with the addition of essential components. In the productions of erythritol and 3-hydroxypropionaldehyde, the replacement with rapeseed meal hydrolysate was not successful.

However, in the production of 1,3-propanediol the strategy was successful. Figure 1 shows an experiment where the proportion of yeast extract was reduced from 5 to 1 g L^{-1} and rapeseed meal hydrolysate (amino nitrogen content of 4 g L^{-1} YE) was added. The maximum productivity almost corresponded to the maximum productivity of the reference with 5 g L^{-1} yeast extract. After a cultivation time of 72 h, 96 g L^{-1} 1,3-propanediol were produced.

Figure 1: Production of 1,3-propanediol using *C. butyricum* with partial (green triangle) and complete substitution of yeast extract (YE) (red asterisk) by rapeseed meal hydrolysate

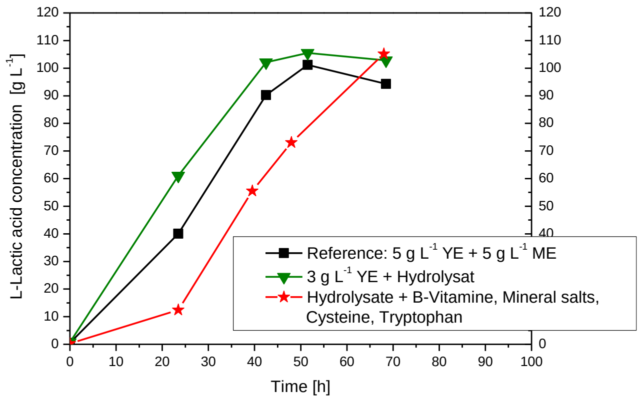


Source: Thünen-Institute/Anja Kuenz (2021).

However, the complete substitution of yeast extract led to a decreased productivity and only 63 g L^{-1} 1,3-propanediol were achieved after 48 hours.

In the production of L-lactic acid the complex nitrogen sources could be completely replaced by rapeseed meal hydrolysate (Figure 2). The lag phases of the three approaches differ, but after about 24 hours the glucose was converted to a final concentration of 105 g L^{-1} L-lactic acid with a comparable maximum productivity, even without any addition of yeast extract or meat extract.

Figure 2: Produktion of L-lactic acid using *L. casei* with partial (green triangle) and complete substitution of yeast extract (YE) and meat extract (ME) (red asterisk) by rapeseed meal hydrolyzate



Source: Thünen-Institute/Anja Kuenz (2021).

Thus, it could be shown that in the production of 1,3-propanediol yeast extract could be replaced for the main part (80%), achieving the same product concentration and maximum productivity. In the production of L-lactic acid, the expensive complex nitrogen sources (yeast extract and meat extract) were completely replaced by the inexpensive, chemically digested rapeseed meal, supplemented with B vitamins, mineral salts, cysteine and tryptophan. The same product concentration of L-lactic acid was achieved with a comparable maximum productivity.

Further information

Contact

[1] Thünen-Institute of Agricultural Technology
Anja.Kuenz@thuenen.de
Ulf.Pruesse@thuenen.de
www.thuenen.de/at

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Publications

Krull S, Brock S, Prüße U, Kuenz A (2020) Hydrolyzed Agricultural Residues—Low-Cost Nutrient Sources for l-Lactic Acid Production. Fermentation 6, 97.

Brock S., Hancock V., Krull S., Kuenz A., Prüße U. (2020) Schlussbericht zum Vorhaben "Substitution von Hefeextrakt in industriell relevanten Bioprozessen (SubBioPro)"; Laufzeit des Vorhabens: 01.05.2017 bis 15.08.2020. Braunschweig: Thünen-Institut, 85 p

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