Accurate amino acid analysis for process design in the cultivation of microorganisms

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Introduction

Cell culture and fermentation broth media are used in the manufacture of biotherapeutics and other biological products. Characterizing the amino acid composition in fermentation broth media is important because deficiencies in these nutrients can reduce desired yields or alter final product quality.
Fermentation broths are complex mixtures of nutrients, waste products, cell debris as well as intermediate products of biosynthesis. There is increased interest in the characterisation of amino acids and their metabolic by-products in fermentation broths, as these components influence the yield of the desired products. In addition to direct uptake for biosynthesis, certain amino acids could be used for energy production under certain conditions.

Another aspect is the optimisation of growth conditions for the cells through a selective supply of individual nutrients. Through the consumption of certain amino acids can lead to shortages, which can fail the consumption of successful fermentation processes. Using amino acid analysis can be performed in such cases a process of optimization. By quantitatively determining the amino acid content in different phases of the process, the consumption of individual amino acids can be depicted and the nutrient medium can be balanced by adding these amino acids accordingly.

Experimental Setup

The fermentation was performed using an EloFerm laboratory fermenter with two identical parallel reactor systems. Each reactor system has a reactor vessel with 1.0 L full volume and has an independent process control.
As a model process, anaerobic fermentation of lactic acid bacterial strain Lactobacillus plantarum strain ATCC 8014 (gram negative, facultative anaerobic) was studied. A pH-controlled (pH=5.6, T= 36°C) and non pH-controlled (final pH of 3.6, T= 36°C) processes were measured and analyzed. From experience, a growth process in the optimal pH range (for this type of bacteria pH 5.6 ÷ 6.0) should guarantee a higher yield and a better vitality status of bacterial cells.

EloFerm, manufactured in Germany is a bench-scaled fermenter package with all necessary features for cultivation, processing and data recording. The intelligent software ensures an easy employment. The control monitor provides various ways of measuring, viewing, storing, comparing and exporting series of cultivation data. EloFerm does contain an implemented inline/online-photometer for continuous recording of the biomass or cell concentration. If you need further information, please do not hesitate to contact us.

The MRS full medium from Carl Roth GmbH was used as the growth medium, which contains, among other things, 10 g/L peptone, 8 g/L meet extract, 4 g/L yeast extract (with a broad spectrum of amino acids, see results) and 20 g/L glucose as a carbon source. The

amino-containing compounds of the MRS medium used are employed for the cultivation of Lactobacillus species as well as for eukaryotic culture systems.

Sample Preparation and Analysis

Before the sample is analyzed in the analyzer, is a simple sample preparation, in the fermentation is negotiating to remove proteins and peptides and was followed by a standard protocol:

400 µL of the sample were mixed with 100 µL precipitation buffer and deposit in the refrigerator for 20 min for the protein precipitation. The sample was centrifuged for 5 min at 14000 rpm. Afterwards the supernatant was filtered with a membraSpin by centrifugation at 14000 rpm for five minutes. 30 µL of the particle free solution were diluted with 70 µL sample dilution buffer and 200 µL sample dilution buffer (including internal standard norleucin 200 nmol/mL)

The samples were analyzed by the Amino Acid Analyzer ARACUS, manufactured and distributed by membraPure GmbH worldwide. ARACUS is using the classic routine analysis of amino acids by post-column derivatization with ninhydrin and the detection at 440 nm and 570 nm.

Figure 1: Amino Acid Analyzer ARACUS

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Table 1: Determined amino acids concentration of an anaerobic fermentation of lactic acid bacterial strain *Lactobacillus plantarum* with and without pH control.

Amino Acid	pH Controlled nmol/mL	pH non-controlled nmol/mL	Δ
P-Ser	333	485.6	-153
Tau	n.q.	n.q.	n.q.
Asp	605.5	723.0	**-118**
Thr	1082	1545.3	**-463**
Ser	76.2	600.6	**-524**
Asn	n.d.	1178.0	n.d.
Glu	3642	4076.0	**-434**
a-AAA	n.d.	n.d.	n.d.
Gly	2447.6	1695.4	+752
Ala	5200	3341.2	+1859
Cit	242	45.2	+197
Val	2232.3	2985.5	**-753**
Cys	108.2	145.8	-38
Met	799.1	< 25	+779
Ile	1527	1934.9	**-408**
Leu	3712.4	5598.7	**-1886**
Tyr	153.6	389.4	-236
Phe	1217.2	2278.9	-1062
g-ABA	1433.3	2845.0	-1412
His	752.3	377.1	+375
Trp	438	277.0	+161

Orn	656.7	115.0	+542
Lys	3355.1	3163.7	+191
NH4	8261.7	11940.7	-3679
Arg	1298.4	2032.9	-735
Hypro	n.d.	291.0	n.d.
Pro	1221.5	681.2	+540

n. q. = not quantified, n.d. = not detected

Conclusion

These results show that ARACUS can be used to determine amino acids in media used for bacterial culture (fermentation broths). High accuracy and robustness of the method are possible with these complex sample matrices. Complex mixtures of amino acids can be monitored during fermentation and provide the analyst with information for process optimisation.

By comparing the two fermentations, the effects of pH control on the individual amino acids can be determined. The differences in the amino acids Thr, Ser, Glu, Val, Asp, Ile and Leu were determined.

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