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Suffer your media from burn-out? - Analyzing stressed media

Did you leave your medium on the bench or have you incubated it for sterility testing before use? Process preparation and surrounding conditions might play a crucial role related to the performance of a medium. Here we present data generated after exposure of media to different conditions other than the cellular influence. The aim was to determine how the induced conditions applied to cell culture media result in molecular changes which in turn might affect the media performance. We incubated medium at 33°C and 37°C in a bioreactor system without cells and exposed it to different light conditions. We observed that the tested conditions can alter culture performances significantly.

RESULTS

Keep the lights off - save energy and your process

Four bottles of cell culture media were exposed to different conditions for 14 days. During that time several samples were taken and 27 amino acids plus 12 vitamins were profiled.

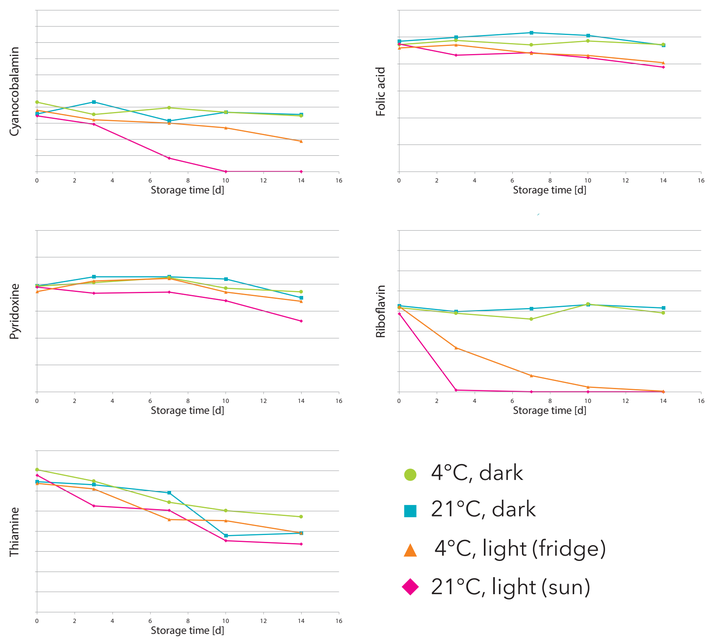

FIG 1: Time-dependent profile of vitamins in media samples at 4°C and 21°C, after light exposure compared to dark storage conditions. Of 39 analytes, only those with differential profiles are depicted.

Cultivations in treated media were carried out to study the effect of light on cell culture growth performance.

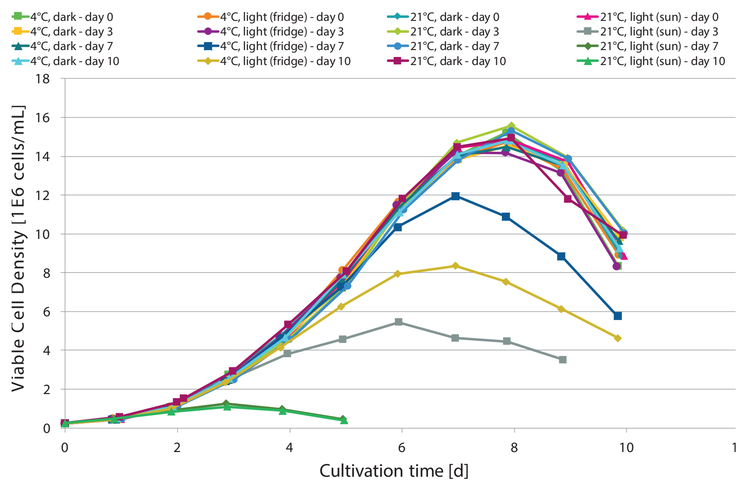

FIG 2: Growth performance of a CHO cell line cultivated in media stored at different light conditions. Storage at 21°C with sunlight treatment induced vast performance limitations starting at day 3. Artificial light at 4°C induced notable performance limitations after 7 days. Storage in the dark did not result in any growth performance deficiencies.

CONCLUSIONS

- Exposure to light alters cell culture media significantly. Next to slight alterations (FIG 1), riboflavin and cyanocobalamin show severe decays. Thiamine was unstable at all conditions.
- Cultivation in light-treated media resulted in reduced growth, increasing with exposure time (FIG 2). Further, notable differences between sun- and artificial light could be detected.
- Non of the analyzed media constituents from the bioreactor tests showed a complete decay. However, accumulation of molecules, such as oxoproline (FIG 3), was observed, which might reduce the growth performance.
- Cysteine is rarely detected in cell culture media. It shows a fast and complete conversion towards cystine in aqueous solution (FIG 5) and can be expected to react even faster in the presence of different molecules in cell culture media.
- Like cysteine, ascorbic acid is another cell culture media ingredient that shows fast decay (oxidation) at all conditions tested (data not shown).

Precultivation - What happens in the reactor stays in the reactor

Bioreactor runs without cells were carried out to test the influence of cultivation conditions on media. No vitamin or amino acid decomposed completely. MS full scans revealed the generation of different molecules over time. One example is shown below.

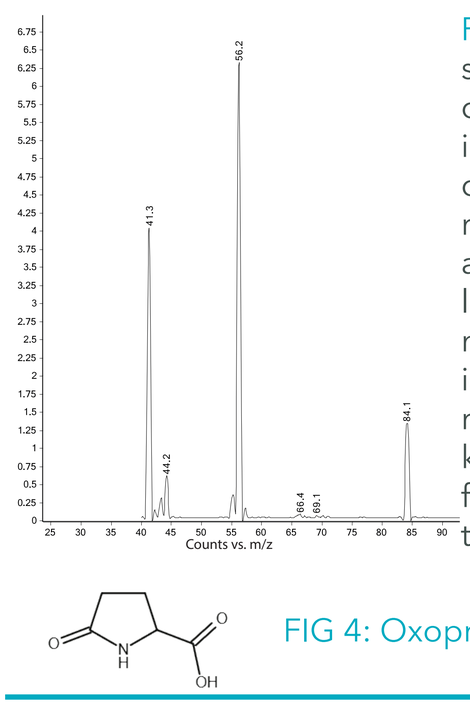

FIG 3: Product ion scan of a molecule first detected in a full scan. It increased in a bioreactor over time and without cellular influence. The molecule could be identified as oxoproline, which is known to originate from glutamine after deamination.

FIG 4: Oxoproline

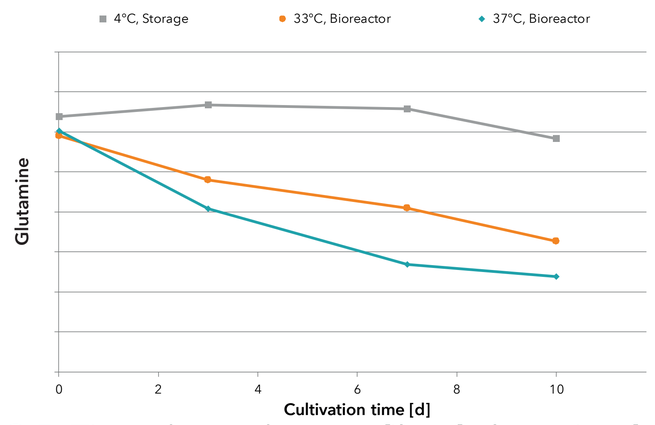

FIG 5: Time-dependent profile of glutamine from media samples at 4°C and cell-free bioreactor samples at 33°C and 37°C.

	Difference Glutamine	Difference Ammonium
10 days, 4° vs 33°	-2.57 mM	+2.54 mM
10 days, 4° vs 37°	-3.46 mM	+3.48 mM

TAB 1: Time-dependent changes of glutamine and ammonium.

Cyst(e)ine stability

Cysteine rapidly oxidizes to cystine, if no reducing conditions are maintained.

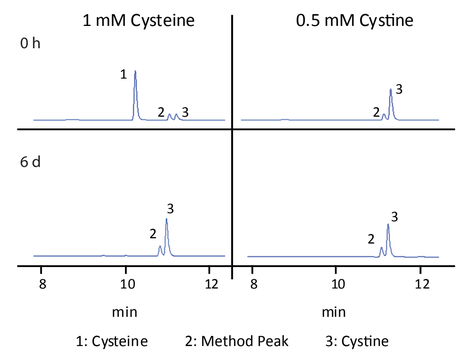

FIG 5: UHPLC chromatograms of aqueous cysteine and cystine solutions. Measured after 0 hours and 6 days of storage.

TAB 2: Time-dependent distribution of cysteine and cystine.

	Duration until sampling	Cysteine (rel. Amt.)	Cystin (rel. Amt.)
1 mM Cysteine	0 h	0,89	0,11
	2 h	0,91	0,09
	5.5 h	0,90	0,10
	24 h	0,65	0,35
	26 h	0,52	0,48
	6 days	0,02	0,98

METHODS

Vitamin analyses Vitamins were measured with Xell's proprietary method *via* LC-MS/MS on a Varian 2000 QQQ coupled to a ProStar HPLC or an Agilent 6470 QQQ coupled to an Agilent 1290 UPLC.

Amino acid analyses Amino acid measurements were carried out with Xell's proprietary method on an Agilent 1290 UPLC. Derivatized amino acids were detected with a DAD.

Cultivation conditions Duplicate batch cultivations of a CHO GS cell line were carried out in stressed vs. reference media in 125 ml flasks (Corning). 37°C, 5% CO_2, 80% humidity and 185 rpm were applied. Cell density and viability were measured on a Cedex automated cell counter.

Molecule identification LC-MS full scans were performed on an Agilent 1290 UPLC coupled to an Agilent 6470 QQQ. Accumulating molecules were identified *via* product scans at different CEs and database comparison of fragments.

Analytical services

For more information regarding Xell's media and spent media analyses please visit:
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