High throughput screening of 1,4-butanediol production in fermentation samples using a LDTD APCI ionization source coupled to a benchtop Orbitrap MS

MP-025

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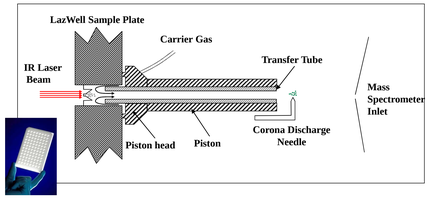
ABSTRACT

1,4-Butanediol (BDO) is an important commodity chemical used to manufacture over 2.5 million tons of valuable polymers annually. It is currently produced exclusively through petrochemical routes from oil and natural gas. A systems-based metabolic engineering strategy was implemented to construct a strain of *E. coli* capable of producing high levels of bio-BDO from renewable carbohydrate feedstocks. To ensure efficient optimization of BDO production, high throughput screening methods to monitor the formation of BDO, key metabolic intermediates and by-products are required. Traditional LCMS approaches cannot sufficiently accommodate throughput demands. In this research, a LTDD APCI source directly coupled to a mass spectrometer (both triple quadrupole and Orbitrap based) was evaluated to fulfill speed and versatility requirements for this application.

METHOD

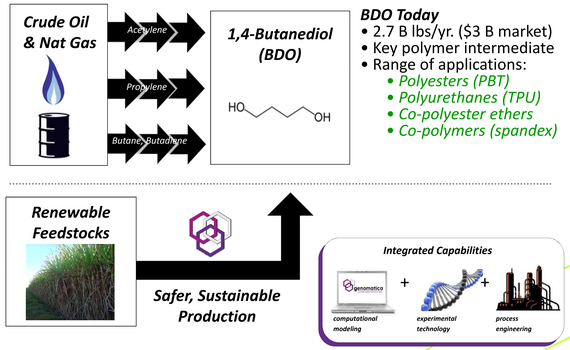
Fermentation broth samples from metabolically engineered E. coli grown on glucose or sucrose as a carbon source were quantified by LC-MS/MS method following their dilution into water. For LDTD analysis, the same set of samples was diluted into a methanol-water mixture (75/25) and a calibration curve was generated from spiking the fermentation medium (for matrix-match) with known BDO concentrations ranging from 1 to 150mM to match the expected sample concentration range. 2 or 3 µL samples were introduced into the LazWell plate followed by LDTD-MS analysis. Both triple quad MS/MS and Orbitrap high resolution accurate mass FTMS data were qualitatively and quantitatively evaluated and compared to LCMS results.

LDTD Operational Principles



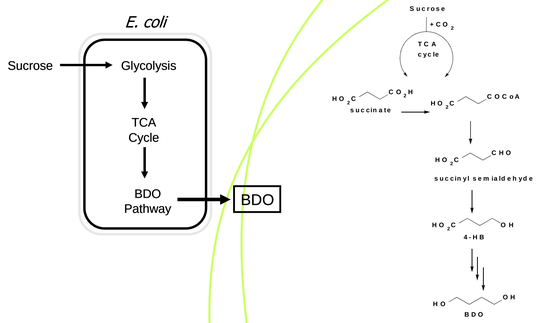
- Samples are dried in standard 96-well plate with a metal sheet insertion
- Thermal desorption induced by a laser at 980 nm (no photon-sample interactions)
- Gaseous neutral species transferred by a carrier gas
- Ionization occurs into the corona discharge region

1,4-Butanediol (BDO) –Today and Tomorrow



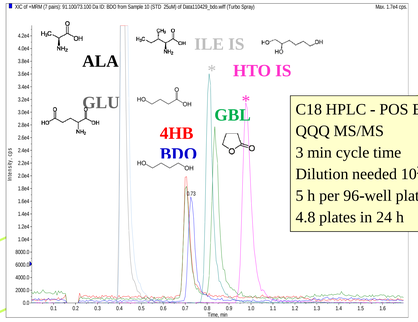
BDO Today
- 2.7 B lbs/yr. ($3 B market)
- Key polymer intermediate
- Range of applications:
  - *Polyesters (PBT)*
  - *Polyurethanes (TPU)*
  - *Co-polyester ethers*
  - *Co-polymers (spandex)*

Sustainable 1,4-Butanediol Bioprocess



High Throughput BDO screening assay is critical
- Gene screening
- Enzyme optimization: Glycolysis, TCA, BDO Pathway
- Strain optimization: BDO tolerance, by-product elimination

Conventional LCMS/MS based analysis of BDO and related metabolites



C18 HPLC - POS ESI
QQQ MS/MS
3 min cycle time
Dilution needed 10^2-10^4 fold
5 h per 96-well plate
4.8 plates in 24 h

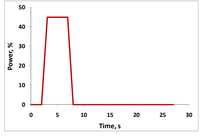
Experimental Setup

- *E.coli* fermentation samples in 96-well plates,19 and 40h time points
- Neat BDO standards 1-150 mM prepared in cell culture medium (M9, MOPS, 5% Glucose, bicarbonate, trace elements, MgSO4, CaCl2 and water)
- All samples & standards were diluted 100-fold in 75% MeOH in water containing 0.5mM ISTD (isotopically labeled 13C-1,4-BDO)
- 3 uL samples & standards were spotted into LazWell sample plate & dried

TSQ Vantage MS/MS: POS & NEG APCI, SRM 91/55 BDO, 90/44 Ala, 148/102 Glu, 103/57 4HB (NEG)

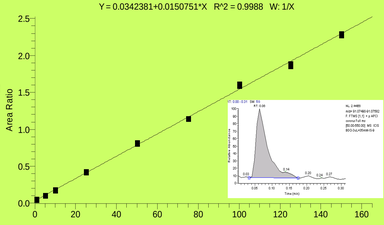
Exactive Orbitrap MS: POS APCI, R=50,000, AGC target 1E6, maximum IT 250ms, 5 ppm m/z window

LDTD T-960 parameters
- **Gas flow at 3L/min**
- **Laser operation :**

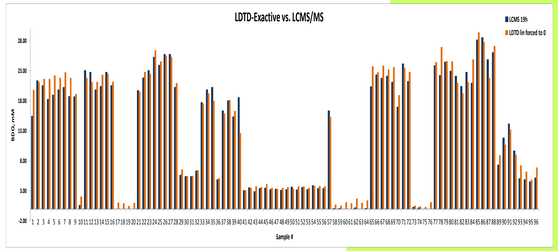


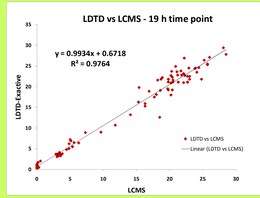
LDTD-Exactive: 1,4-BDO calibration curve

- 1 – 150 mM dynamic range
- 100-fold dilution in MeOH/H2O
- 3uL sample volume
- 13C-BDO as internal standard (0.5mM in MeOH/H2O diluent)
- Excellent linearity $R^2 = 0.9988$
- **ISTD is absolutely critical!!!**
- **Stdev of ISTD peak area could reach 40-50% across 96-well plate on average**



LDTD-Exactive *vs.* LCMS/MS BDO quantitation - 19h fermentation time point





CV < 15% (average IS)

Conclusions

- High throughput quantitative assay of 1,4-BDO in fermentation and cell culture samples has been developed based on LDTD-MS coupling. Both TSQ MS/MS and high resolution benchtop Orbitrap instruments have been tested.
- Good correlation between LDTD and conventional LCMS/MS methods has been obtained ($r^2 > 0.97$, CV below 15 %).
- Sample matrix matching and good internal standard are critical to achieve accurate quantitation.
- Throughput of BDO screening has been increased almost an order of magnitude, from 3min to 20s per sample → 35 min analysis time per 96-well plate.
- Application of LDTD - benchtop Orbitrap coupled system to quantitatively screen fermentation samples in high throughput manner for 1,4-BDO production has been demonstrated. High resolution and mass accuracy of Exactive enabled fast BDO screening in complex matrices, as well as monitoring and/or finding other metabolites of interest, with minimal method development and sample preparation.