**Improving product specificity of whole-cell alkane oxidation in non-conventional media: A multivariate analysis approach**

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**Highlights**

* Polarity of co-solvents determines product specificity.
* Accumulation of alcohol over acid using more polar solvents.
* PLS model showed defining factors are solubility parameters

**1. Introduction**

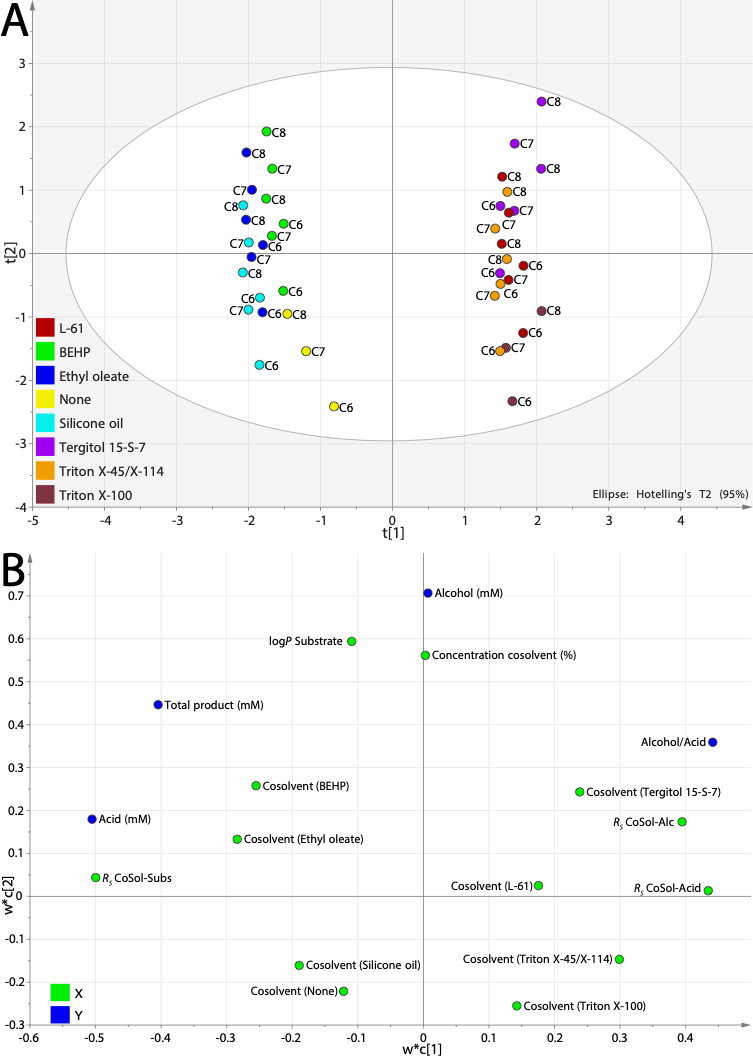
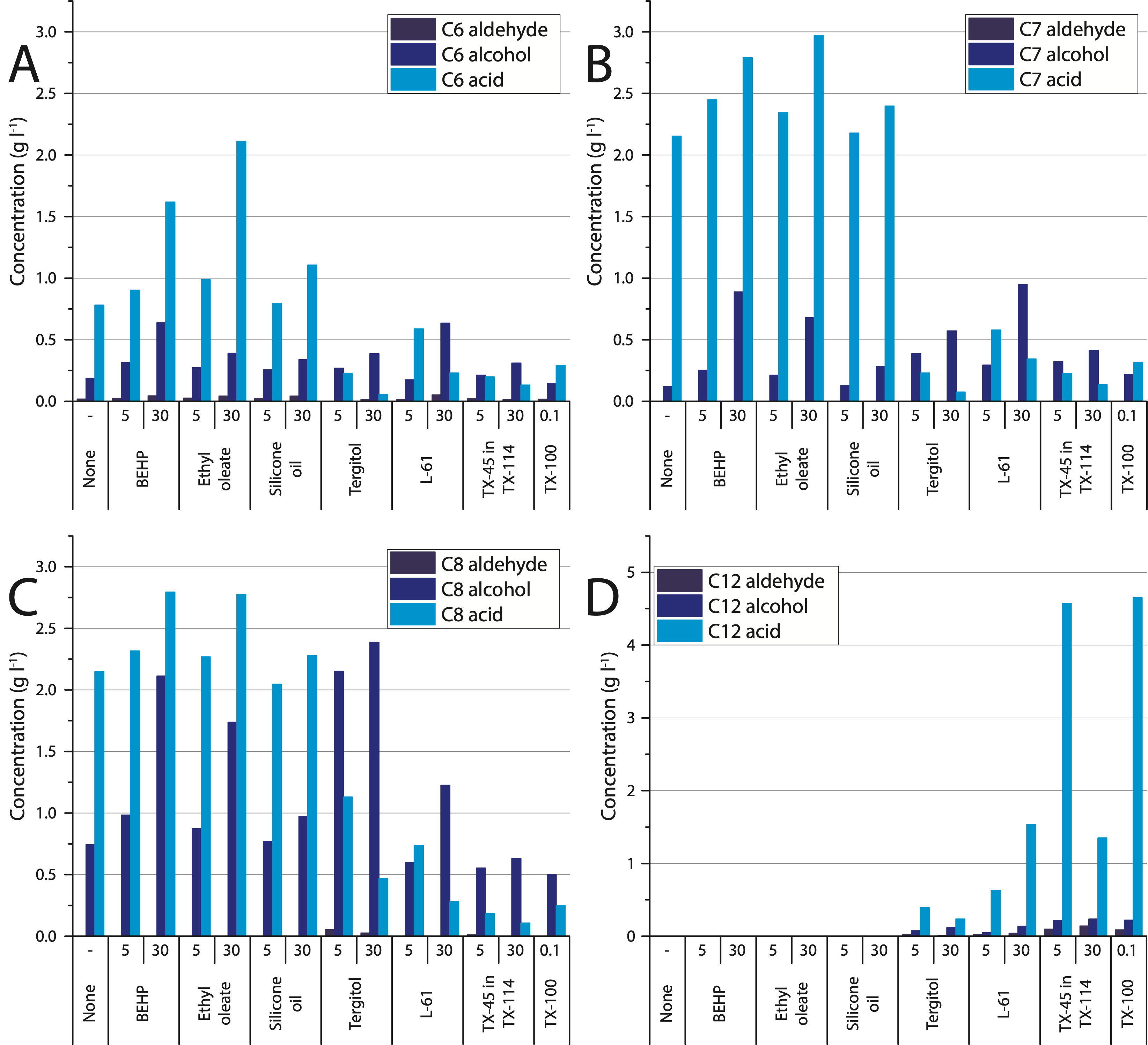
Two-liquid phase reaction media have long been used in bioconversions to supply or remove hydrophobic organic reaction substrates and products to reduce inhibitory and toxic effects on biocatalysts [1]. In case of the terminal oxyfunctionalisation of linear alkanes by the AlkBGT monooxygenase the excess alkane substrate is often used as a second phase to extract the alcohol, aldehyde and acid products [2]. However, the selection of other carrier phases or surfactants is complex due to the large amount of parameters that are involved, such as: biocompatibility, substrate bioavailability and product extraction selectivity.

This study investigates co-solvents of different polarities and structures as secondary solvents for the whole-cell alkane bio-oxidation by AlkBGT. Initially, the impact of six co-solvents at two concentrations is studied. Particular focus is on the overall product yield and specificity of the bio-oxidation of four linear alkane substrates. In order to efficiently screen this wide range of experimental conditions, a high-throughput microwell platform specifically customised for non- conventional media is used [3]. In a second step experimental data is combined with estimated physicochemical properties of the co-solvents in a multivariate Partial least squares projections to latent structures (PLS) regression analysis. This allows the identification of key properties of co-solvents that specifically affect the AlkBGT reaction in terms of product specificity and yields.

**2. Methods**

Materials and methods for whole cell bioconversion in customized microwell plates and analysis of the reaction products by gas chromatography has been described in detail in Kolmar et al 2018 [3]. For data analysis physicochemical properties i.e. Hansen and logP parameters were estimated for co-solvents and reaction substrate and products using COMSOquick software. PLS regression analysis was performed (SIMCA 13.0.3, Umetrics) for analysing multiple variables in one model.

**3. Results and discussion**

Partial least square regression showed that the defining factors for product specificity are the solubility properties of reaction substrate and product in the co-solvent, as measured by Hansen solubility parameters. Thus the polarity of co-solvents determines the accumulation of either alcohol or acid products. Whereas usually the acid product accumulates during the reaction, by choosing a more polar co-solvent the 1-alcohol product can be accumulated. Especially with Tergitol as co-solvent, a 3.2 fold improvement in 1-octanol yield to 2.4 g l−1 was achieved relative to the control reaction without co-solvents (Figure 1).

**Figure 1.** PLS model M2 score scatter plot of response data showing X-scores (t) of the first component along the x-axis and X-scores of the second along the y-axis (A) Co-solvent screening with octane substrate after 24h at 30°C and 250rpm, at varying co-solvent percentages in substrate indicated below x-axis (C).

**4. Conclusions**

The application of co-solvents is a promising strategy to influence whole-cell alkane oxidations. Further work needs to investigate efficient downstream processing options for the most promising candidates to fully leverage their advantages.

**References**

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