

Review of: "Rational Protein Engineering of Bacterial N-Demethylases to Create Biocatalysts for the Production of Methylxanthines"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

The authors synthesized 1-methylxanthine and paraxanthine via biological route with the use of catalysts Ndm (A-D), which is worthy of publication. Below are my recommendations:

Lines 142-148

Include brand/model/city/country of equipment and supplies used.

How was the elution? Isocratic or gradient? Include the information.

Insert the volume injected and how the samples were prepared for analysis.

Insert which standards were detected in samples and the concentration range used for quantification.

Include a chromatogram to represent the standards and/or sample as well identifying each compound.

Lines 150-156: Include/discuss any result from you which may justify this statement. Sound general.

Table 1:

I recommend the authors modify, because sound confusing the way the data are presented. For instance, the paraxanthine synthesized is attributed to caffeine conversion, and the 1-methylxanthine to the theophylline conversion, right? If yes, I recommend present the data in separate tables

Also, I did not get the term "percent of... in product". Does it represent the % of material converted into products? It is not clear.

Fig 2A // L174-175:

How the enzymatic activity decreased in FIG 2A? The control decreased with time, so what did the authors expect here?

Explain which parameter explained in Table 1 highlights the decreasing of enzymatic activity. I saw that yield of methylxanthines converted were lower with DdA

Figures captions: The way they were written is confusing; rewrite the captions and clearly identify the differences within the treatments

Figure 2:

Since all symbols are different the authors should insert all in the same color, otherwise looks confusing at first.

