Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary information

Sustainable bioproduction of the blue pigment indigoidine: Expanding the range of heterologous products in *Rhodosporidium toruloides* to include non-ribosomal peptides

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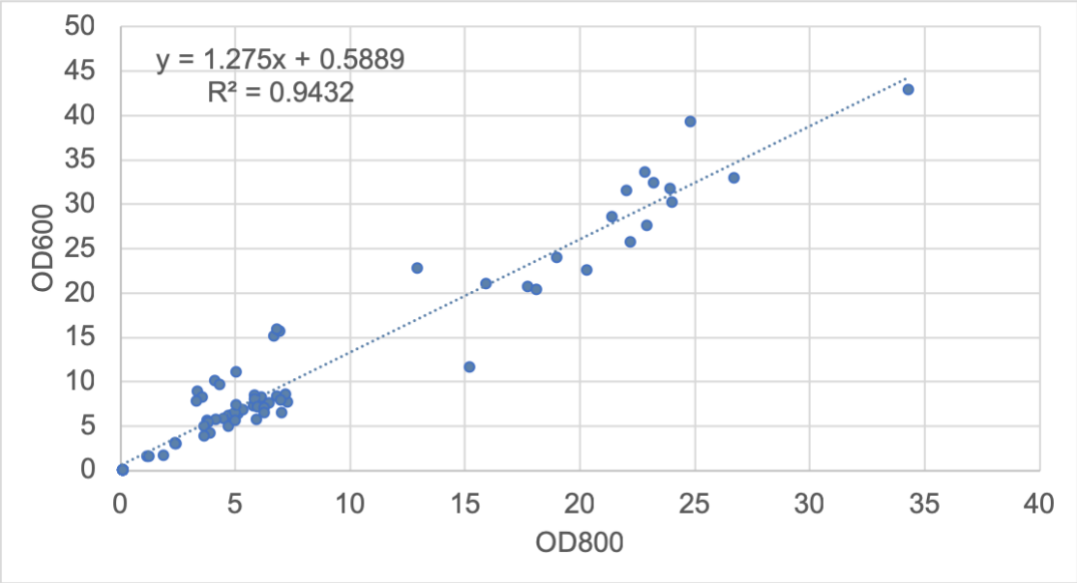


Fig. S1 Correlation of OD_{800} and OD_{600} measurements.

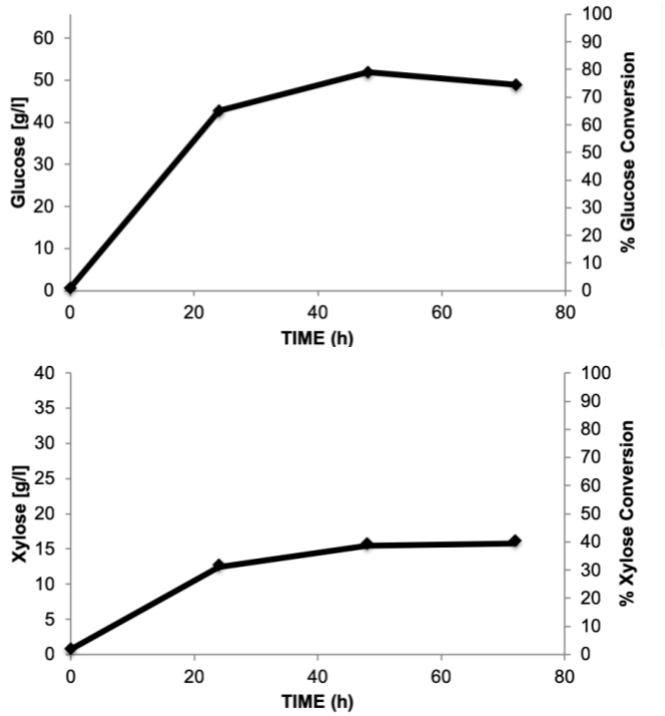


Fig. S2 Sugar conversion during pretreatment process of sorghum biomass.

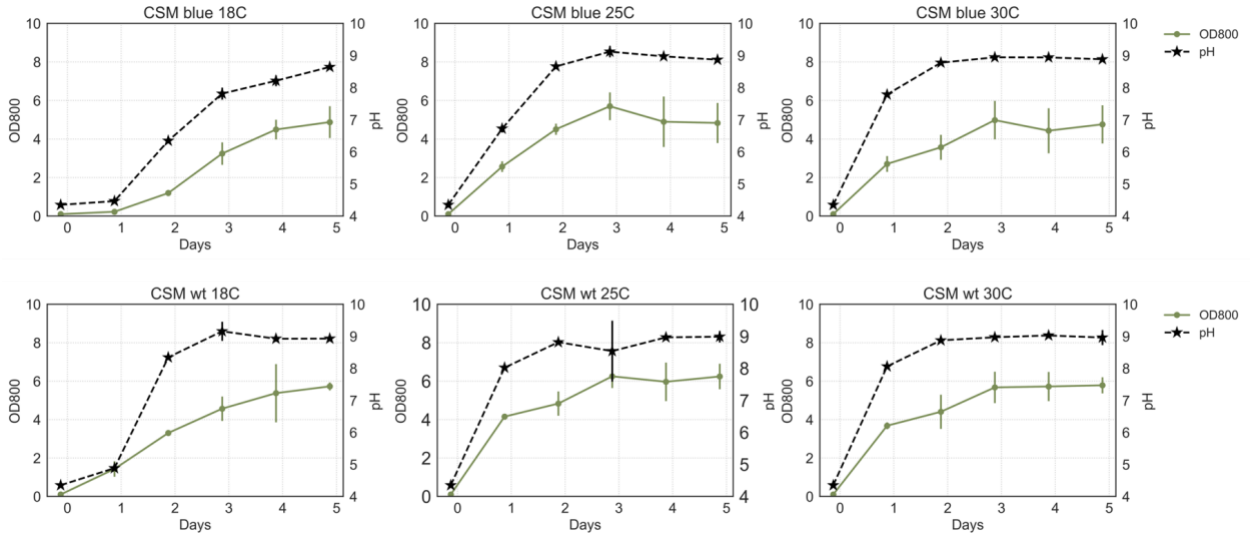


Fig. S3 Growth profiles of BlueBelle (top) and IFO0880 (wt, bottom) in synthetic defined media cultivated at different temperatures. OD_{800} values, depicted in green, and the culture pH shown as black rhombus, are plotted against time. Error bars represent SD of triplicates.

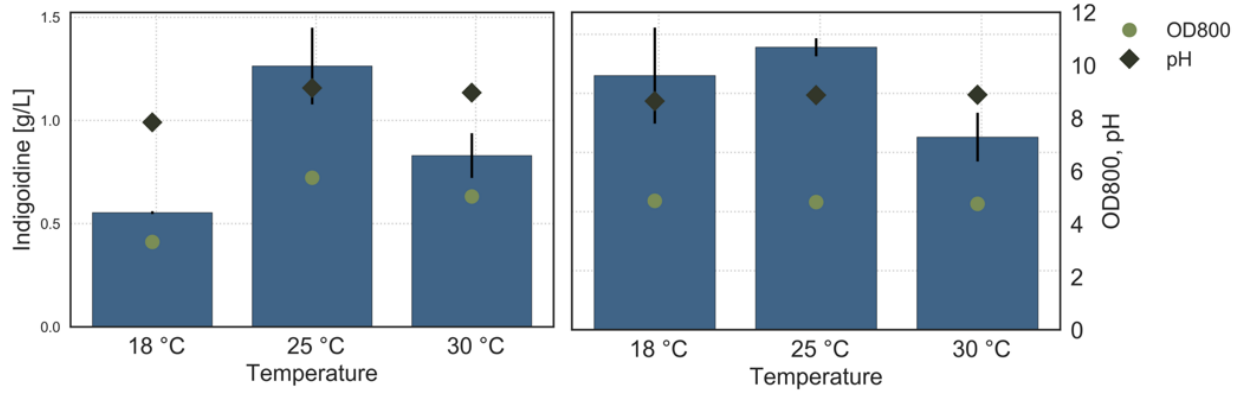


Fig. S4 Production profile of indigoidine cultures after 3 days (left) and 5 days (right) of cultivation at different temperatures. Indigoidine concentrations (blue bars) OD_{800} values (green dots) and culture pH (black rhombus) were measured. Error bars represent SD of 3 replicates.

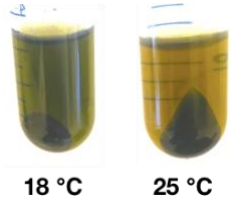
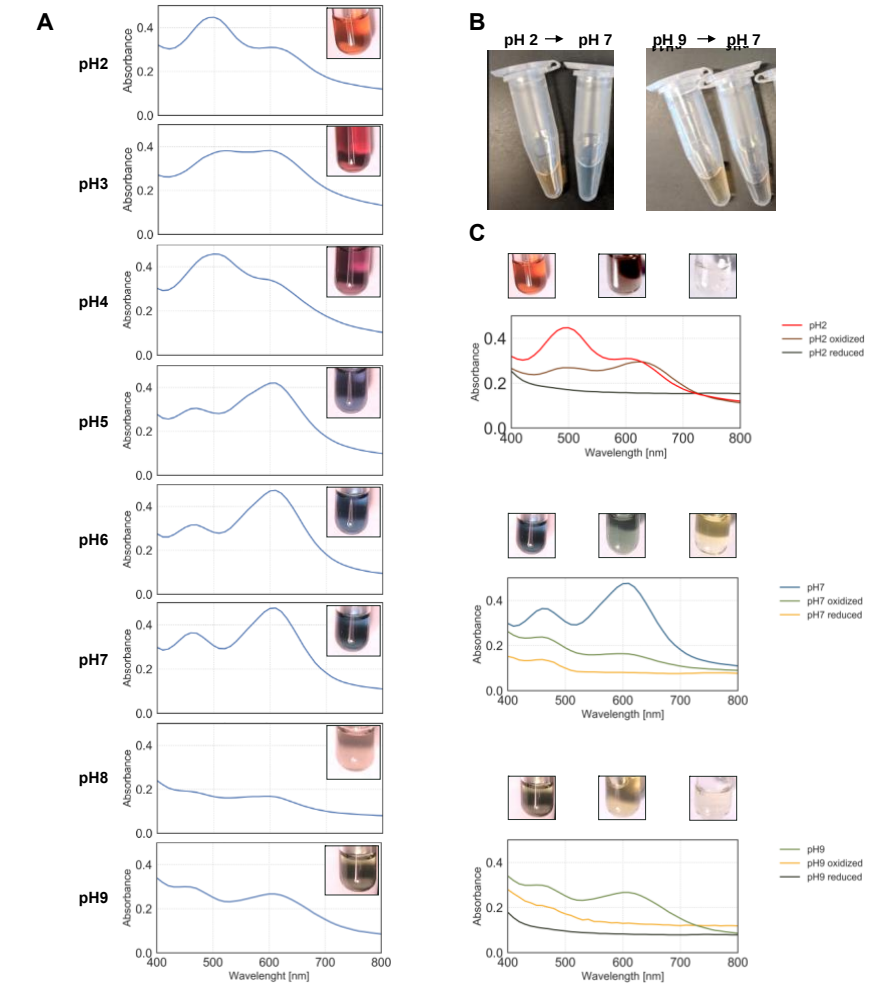


Fig. S5 Coloration of supernatant after 5 days of cultivation at two different temperatures.


A
pH2
pH3
pH4
pH5
pH6
pH7
pH8
pH9
Absorbance
Wavelenght [nm]
B
pH 2 → pH 7
pH 9 → pH 7
C
pH2
pH2 oxidized
pH2 reduced
pH7
pH7 oxidized
pH7 reduced
pH9
pH9 oxidized
pH9 reduced
Wavelength [nm]

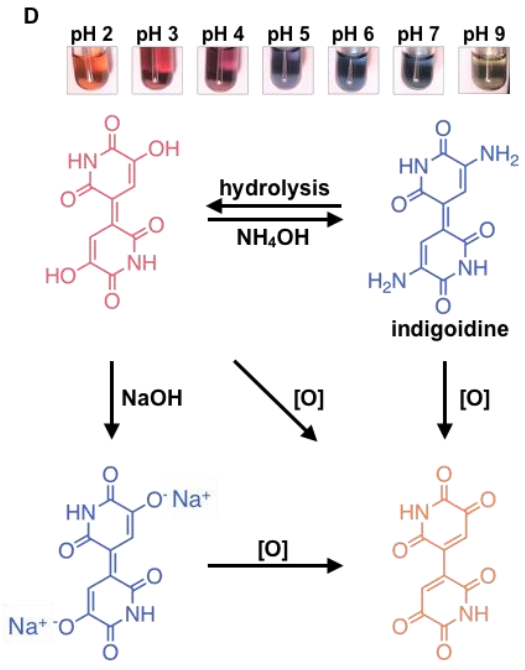
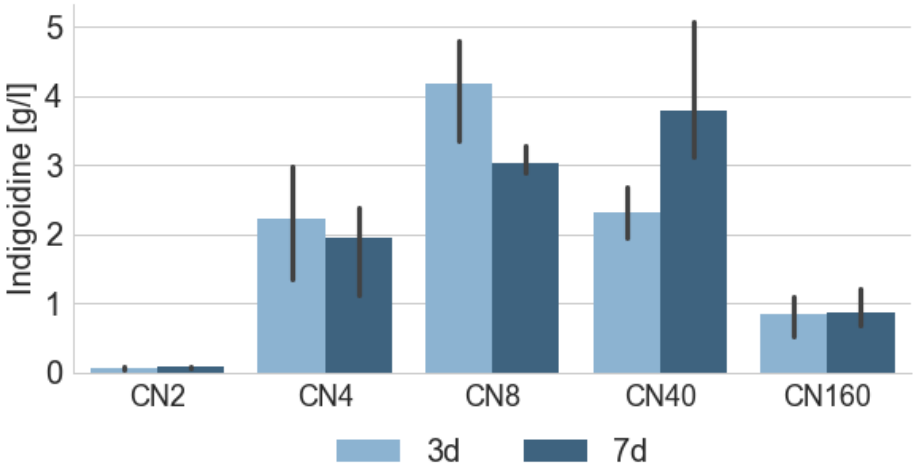

D
pH 2
pH 3
pH 4
pH 5
pH 6
pH 7
pH 9
hydrolysis
NH4OH
indigoidine
NaOH
[O]
[O]
[O]

Fig. S6 Effect of pH and redox state on hue of the solution. **A** UV-Vis spectra of indigoidine solutions with pH ranging from 9 to 2 in intervals of 1. **B** Re-adjustment of the pH from acidic (pH 2) and alkaline (pH 9) to neutral (pH 7). **C** UV-Vis spectra of indigoidine solutions at pH 2, 7 and 9 after oxidation with hydrogen peroxide and reduction with dithionite, respectively. **D Top** The solution of indigoidine displays an increasingly intense hue of red when the pH decreases. Representative solutions are shown. **Bottom** Structural derivatives of indigoidine observed in this study shown in corresponding colors. Indigoidine can undergo hydrolysis to yield a red pigment, hydroxyidigoidine ((*E*)-5,5'-dihydroxy-2*H*,2'*H*-[3,3'-bipyridinylidene]-2,2',6,6'(1*H*,1'*H*) -tetraone, top left), which can react with NH_4OH to obtain indigoidine reversibly. Alternatively, hydroxyidigoidine can be deprotonated by NaOH and form a blue alkali metal adduct (bottom left). In the presence of air, any form of indigoidine (*i.e.* indigoidine and hydroxyidigoidine) can be oxidized to form a ketone, [3,3'-bipyridine]-2,2',5,5',6,6'(1*H*,1'*H*) -hexaone (bottom right), with a characteristic orange color. The depiction of structures is adapted from Kuhn *et al*. [11]



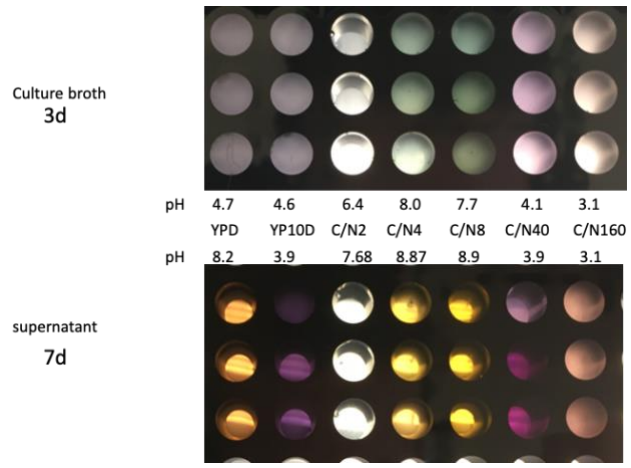


Fig. S7 Top Impact of C/N ratio on the indigoidine production and culture pH after 3 (light blue) and 7 days (dark blue) of cultivation using 100 g/L glucose and varying amounts of urea as carbon and nitrogen source respectively. Error bars represent SD of 3 replicates. **Bottom** Images of the culture broth after 3 days and the supernatant of the cultures after 7 days.

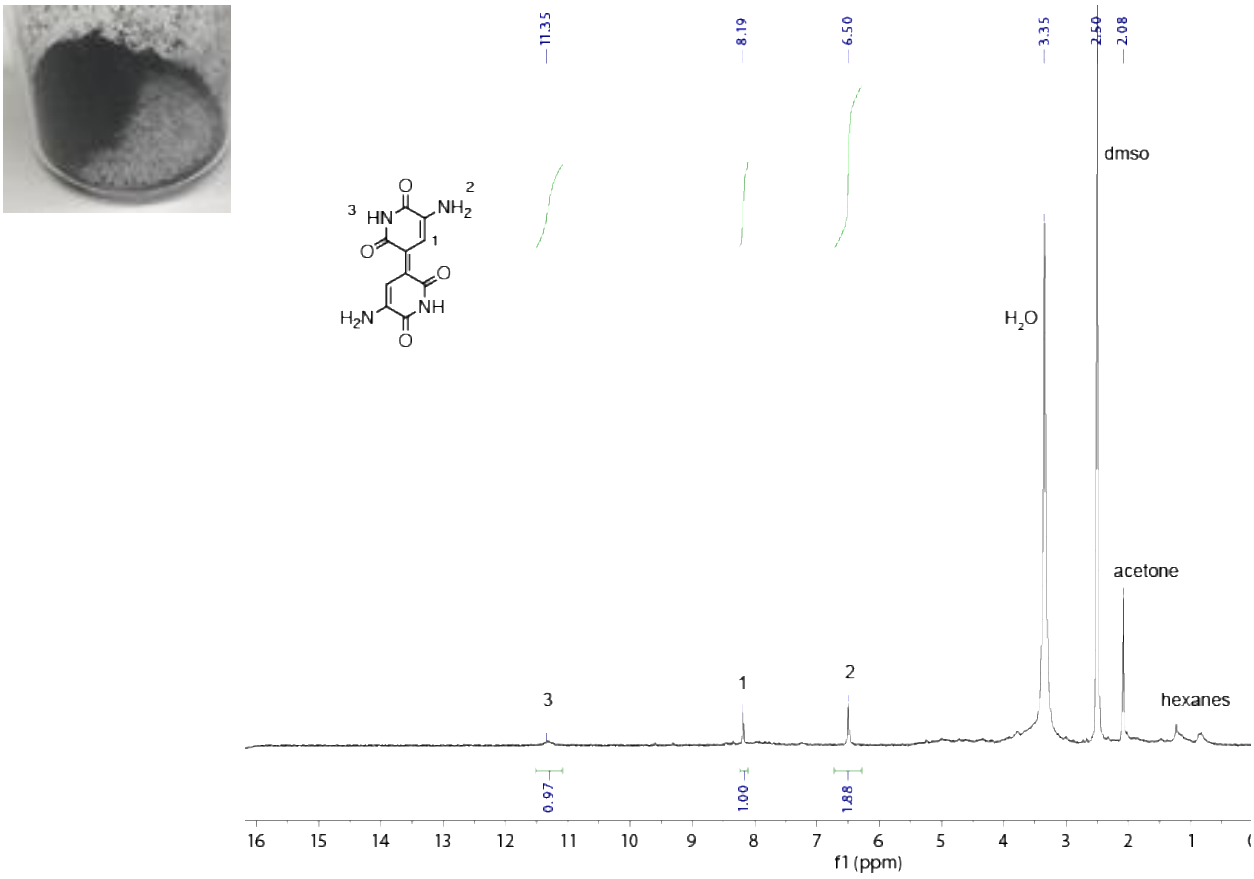


Fig. S8 Left After extraction and purification of indigoidine as described in the Experimental section, a dry powder was obtained. The picture shows the final product (400 mg dry Indigoidine) extracted and purified from 400 mL culture broth from a shake flask experiment using glucose as carbon source after 2.5 days at a concentration of 2 g/L. **Right** NMR analysis was performed to confirm the chemical structure and purity of the compound. Acetone and hexanes were used in the washing process.

Table S1 Normalized Nitrogen sources

The amount of nitrogen sources was calculated to the equivalent of 1.06 g nitrogen/L. For the complex nitrogen sources the following assumptions were made in agreement with manufacturer's description: Nitrogen content of Yeast extract = 10.9% (BD), peptone= 16% and of soy peptone = 8.1%.

Nitrogen Source	Amount
Ammonium sulfate	5 g/L
Urea	2.27 g/L
Glutamine	5.53 g/L
Glutamate	11.12 g/L
Potassium nitrate	7.62 g/L
Yeast extract	9.71 g/L
Peptone	6.62 g/L
Soy Peptone	13.07 g/L