Institut für Lebensmittel- und Umweltforschung e.V. (ILU)
Kontakt: Martin Almendinger | Papendorfer Weg 3 | D-14806 Bad Belzig | www.ilu-ev.de | martin.almendinger@ilu-ev.de

Heterotrophic cultivation of *Galdieria sulphuraria* under non-sterile conditions in digestate and hydrolyzed straw

Daniel Pleissner [1,2], *Astrid Victoria Lindner* [2], *Nicole Händel* [1]

[1] ILU Institute for Food and Environmental Research, 14806 Bad Belzig, Germany, [2] Leuphana University Lüneburg, 21335 Lüneburg, Germany

Introduction

Waste streams from food and agriculture processing appear in considerable amounts globally. To the 1.3 billion tons of food wasted annually come in addition billion tons of agricultural residues of non-edible lignocellulosic biomass (e.g., straw) as well as liquid waste streams (e.g., digestate and wastewater) with a high nutrient load. The aim of this study was an investigation of the cultivation of *Galdieria sulphuraria* in presence of nutrients recovered from digestate obtained after anaerobic digestion of cattle manure as well as straw after hydrolysis. Particular attention has been paid on the non-sterile cultivation of *G. sulphuraria* to provide an approach, which not only allows an efficient use of waste streams but is also simple enough to be implemented decentralized in rural areas.

Characterization of digestate and straw
↓
Enzymatic digestion using proteases and/or cellulases
↓
G. sulphuraria cultivation in digestate (N source) and straw hydrolysate (C source)
↓
Characterization of algal biomass: protein, lipid, starch content

Results and Discussion

Digestate: Assumption: Complex composition inhibits growth of *G. sulphuraria.* Approach: Cultivations in flasks with different concentrations of hydrolyzed or untreated digestate (Figures 1 and 2), cyanidium medium was used as control.

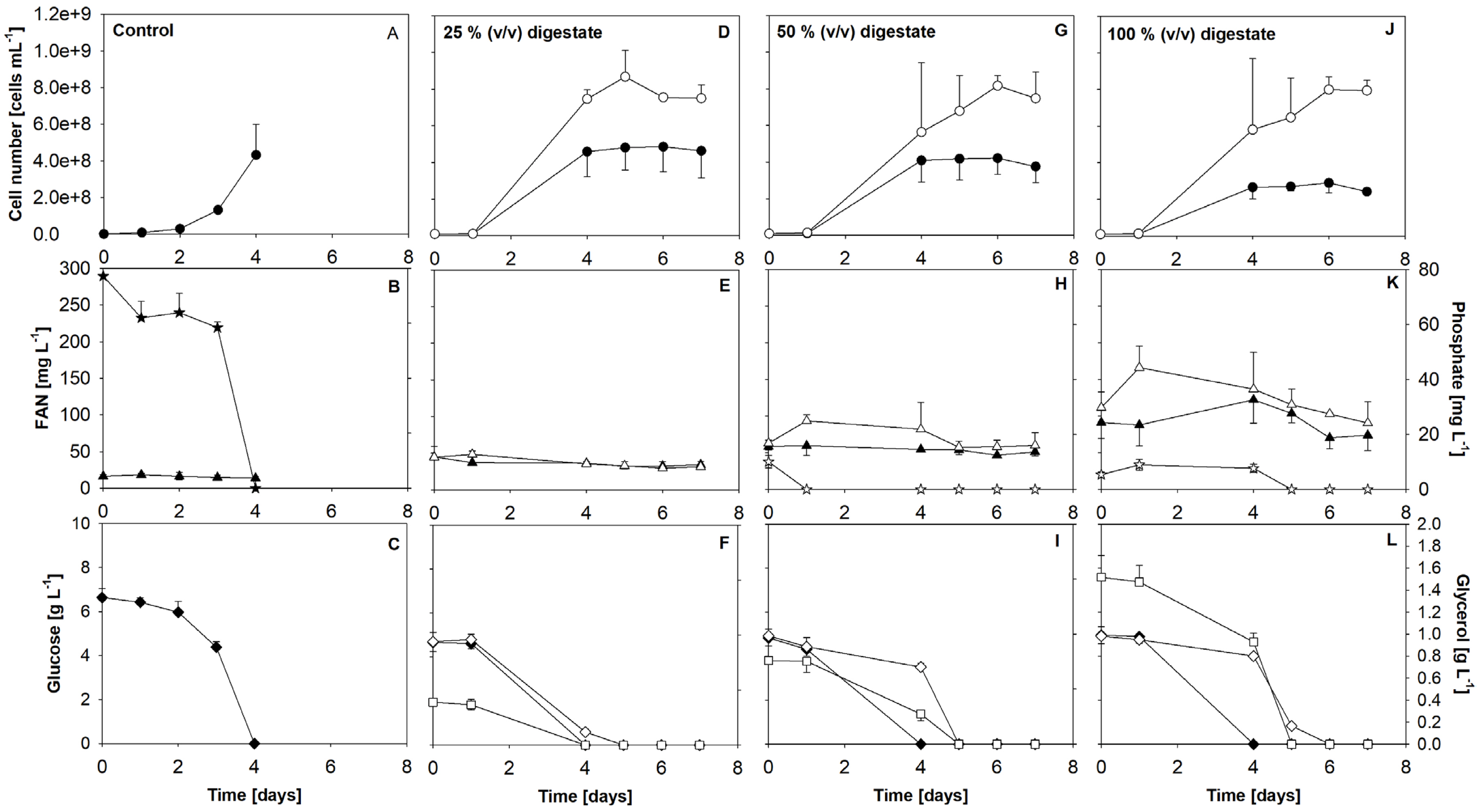

Figure 1: Time profiles of cell number (circle), free amino nitrogen (triangle, FAN), phosphate (star), glucose (diamond) and glycerol (square) concentrations in flask cultures of *G. sulphuraria* grown in triplicate in cyanidium medium as control (A-C), and digestate of different concentrations (25 %, D-F, 50 %, G-I, and 100 %, J-L, v/v). The digestate solutions were either used after treatment with Protease S-02 (open symbol) or untreated (closed symbol).

Exponential growth in all cultivations from day 1 to day 4.

Control (Figure 1A-C):

6.5 g L^{-1} glucose, 80 mg L^{-1} phosphate almost fully consumed after 4 days; 5×10^{8} cells mL^{-1}, μ = 1.2 day^{-1}.

Untreated digestate (Figure 1D-L, closed symbols):

Glucose and phosphate limitations leads to stationary phase after day 4.

Increasing digestate concentration leads to decreasing max. cell concentration (5×10^{8} to 3×10^{8} cells mL^{-1}).

$\mu_{25\%} = \mu_{50\%} = 1.0\ day^{-1}$, $\mu_{100\%} = 0.9\ day^{-1}$

Treated digestate (Figure 1D-L, open symbols):

Increased growth rates and max. cell concentrations, due to enhanced accessability of FAN.

Figure 2: Applied substrates: Straw and digestate.

Straw hydrolysate as alternative carbon source

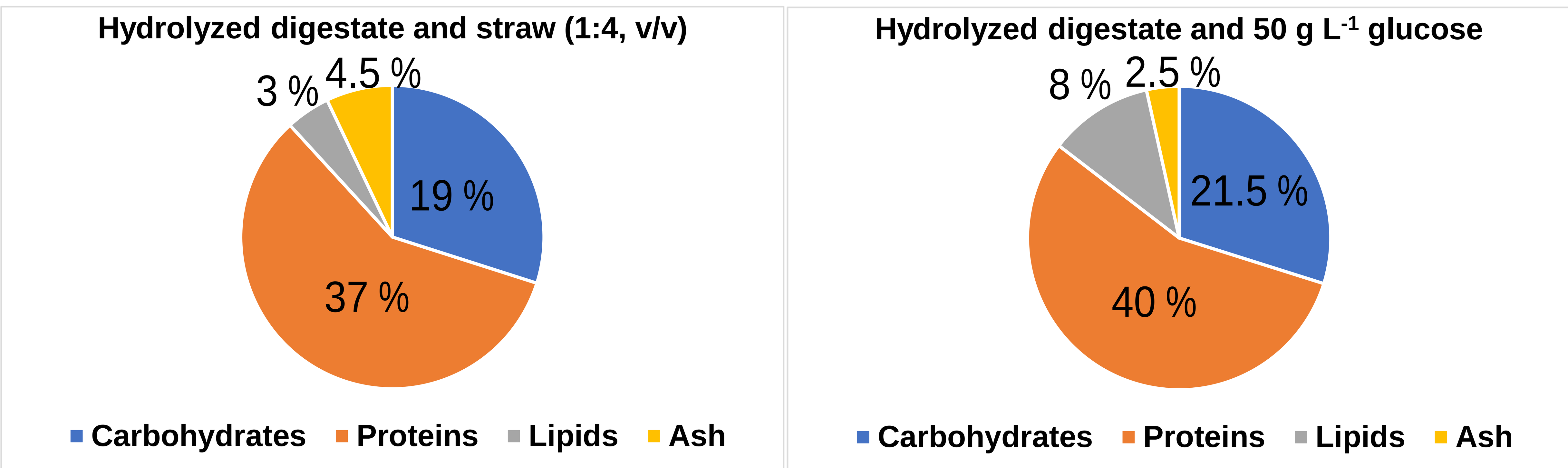

Figure 3: Biomass composition of produced *G. sulphuraria* biomass.

All cultivations resulted in average growth rates of 0.8 day^{-1}.

Hydrolzed straw is a feasible carbon source for *G. sulphuraria*.

Biomass had a protein content of around 40 % (w/w), followed by carbohydrates of around 20 % (w/w) and lipids with 3-8 % (w/w).

Conclusions

This study revealed the potential of *G. sulphuraria* to utilize agricultural residues and to form biomass with a protein content of around 40% (w/w). Considering the possibilities to implement decentralized processes, *G. sulphuraria* can contribute to add value to straw and digestate in rural areas. This approach makes residue utilization not just more economically attractive, but also presents new opportunities for feedstock production for food, fine chemical, pharma and material sectors.

Further information is available here: Pleissner D, Lindner AV, Händel N. 2021. Heterotrophic cultivation *of Galdieria sulphuraria* under non-sterile conditions in digestate and hydrolyzed straw. Bioresource Technology, 337, https://doi.org/10.1016/j.biortech.2021.125477.
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