Supplementary Materials

Process design and carbon footprint analysis of thraustochytrid biomass production using distillery wastewater

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ESTIMATION OF CULTIVATION REACTIONS AND CONVERSIONS

The assumed balanced model equation for thraustochytrid cultivation is as follows:

$$0.9CH_{1.57}O_{0.31}N_{0.29} + 2C_6H_{12}O_6 + 0.08O_2 \longrightarrow 9CH_{2.58}O_{0.23}N_{0.03}S_{0.002} + 4.4CO_2 + 1.7H_2O \quad \text{(S1)}$$

The basis for calculating the yield coefficients for each compound came from the chemical oxygen demand (COD) of the distillery wastewater before and after thraustochytrid cultivation. COD reduction in DWW was assumed to represent total glucose consumption for the production of biomass.

Supplementary Table 1. COD values of DWW before and after TB cultivation

Parameter	Value
Before cultivation, mg/L	72241
After cultivation, mg/L	8582
COD reduced, g/L	63.66

COD reduction, %	88.12
Glucose conversion, %	88.12

The yield coefficient for glucose to biomass ratio was determined using the values of biomass yield during the start and end of cultivation. The product yield coefficients were obtained from the following formula:

$$y_{product} = {}^{g_{product}}/_{g_{glucose}} \quad \text{(S1)}$$

Supplementary Table 2. TB yield obtained during initial and final phases of cultivation

Parameter	**Value**
Initial biomass yield, g/L	2.535
Final biomass yield, g/L	7.65
Total biomass yield, g/L	5.115
COD reduced, g/L	63.66
Yield coefficient of biomass, g/g	0.0803

Supplementary Table 3. Summary of yield coefficients

Parameter	**Value**
Glucose conversion, %	88.12
$y_{biomass}$	0.0803
y_{CO_2}	0.5338
y_{O_2}	0.0067
y_{H_2O}	0.0859

PYTHON SCRIPT FOR CUSTOM BIOREACTOR UNIT

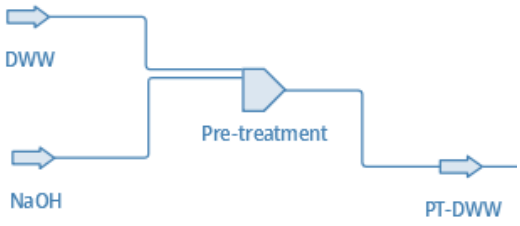
The Python script for the custom unit was interpreted using IronPython. The script was based from the study of Sreemahadevan *et al.* (2024)[1].

```
import clr
from System import Array

# Get input streams
feed1 = ims1
feed2 = ims2

# Get properties
T = ims1.Phases[0].Properties.temperature
P = ims1.Phases[0].Properties.pressure
n = int(feed1.GetNumCompounds())

# Get mass flows
inflows1 = list(feed1.GetProp("flow", "Overall", None, "", "mass"))
inflows2 = list(feed2.GetProp("flow", "Overall", None, "", "mass"))

# Combine streams
outflows = [0.0] * n
for i in range(n):
    outflows[i] = inflows1[i] + inflows2[i]

# define component indices
glucose_index = 2
o2_index = 12
co2_index = 8
water_index = 9
biomass_index = 15

# reaction parameters
glucose_conversion = 0.8812
```

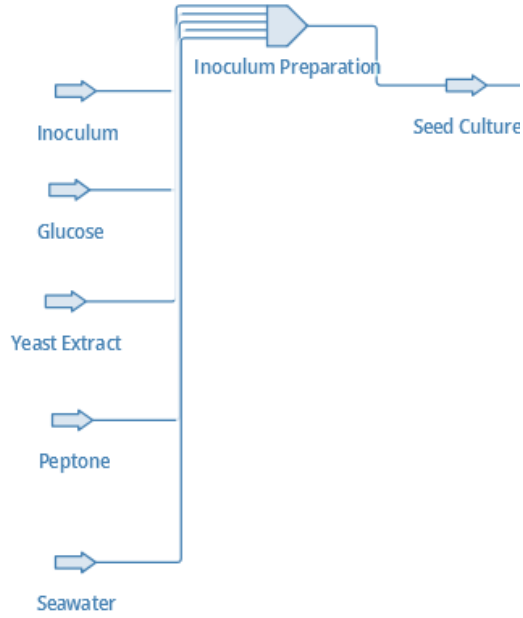
```
Y_biomass = 0.08034999
Y_co2 = 0.533758048
Y_o2 = 0.006688198
Y_water = 0.085920186

# reaction calculation
glucose_consumed = outflows[glucose_index] * glucose_conversion
o2_required = glucose_consumed * Y_o2

# Check oxygen limitation
if outflows[o2_index] < o2_required:
    glucose_consumed = outflows[o2_index] / Y_o2

# Update component flows
outflows[glucose_index] = outflows[glucose_index] - glucose_consumed
outflows[biomass_index] = outflows[biomass_index] + (glucose_consumed *
Y_biomass)
outflows[co2_index] = outflows[co2_index] + (glucose_consumed * Y_co2)
outflows[o2_index] = outflows[o2_index] - (glucose_consumed * Y_o2)
outflows[water_index] = outflows[water_index] + (glucose_consumed * Y_water)

# Calculate total flow
wf = sum(outflows)

# Calculate mass fractions
massfrac = [0.0] * n
for i in range(n):
    if wf > 0:
        massfrac[i] = outflows[i] / wf
```

```
    else:
        massfrac[i] = 0.0

# Convert to .NET array
xwoarr = Array[float](massfrac)

# Get property package and convert mass to mole fractions
pp = feed1.PropertyPackage
if pp is not None:
    xmo = pp.AUX_CONVERT_MASS_TO_MOL(xwoarr)
else:
    # If no property package, use mass fractions directly (not ideal but works)
    xmo = xwoarr

# Set output stream
oms1.SetProp("fraction", "Overall", None, "", "mole", xmo)
oms1.Phases[0].Properties.temperature = T
oms1.Phases[0].Properties.pressure = P
oms1.Phases[0].Properties.massflow = wf
```

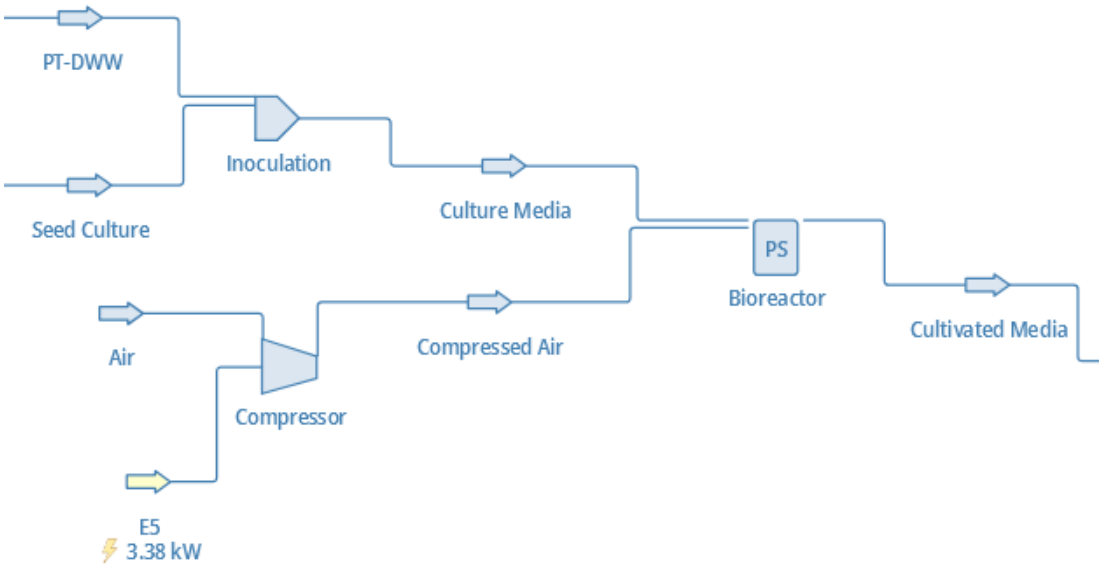
PROCESS FLOWSHEET



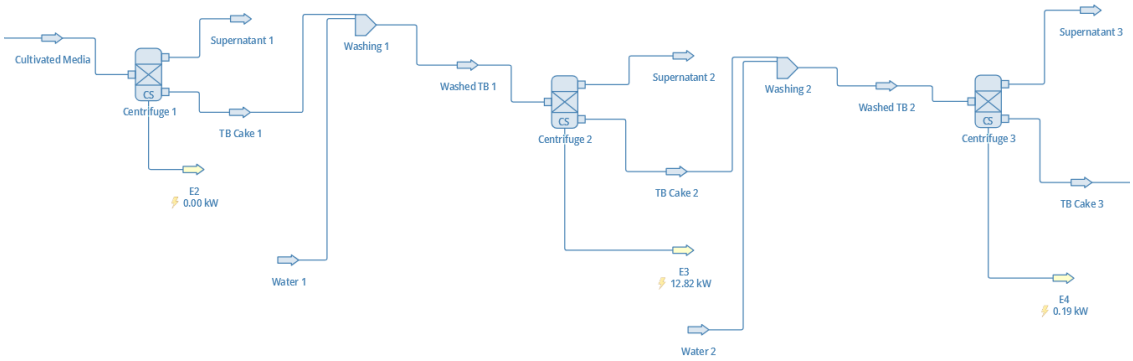
A



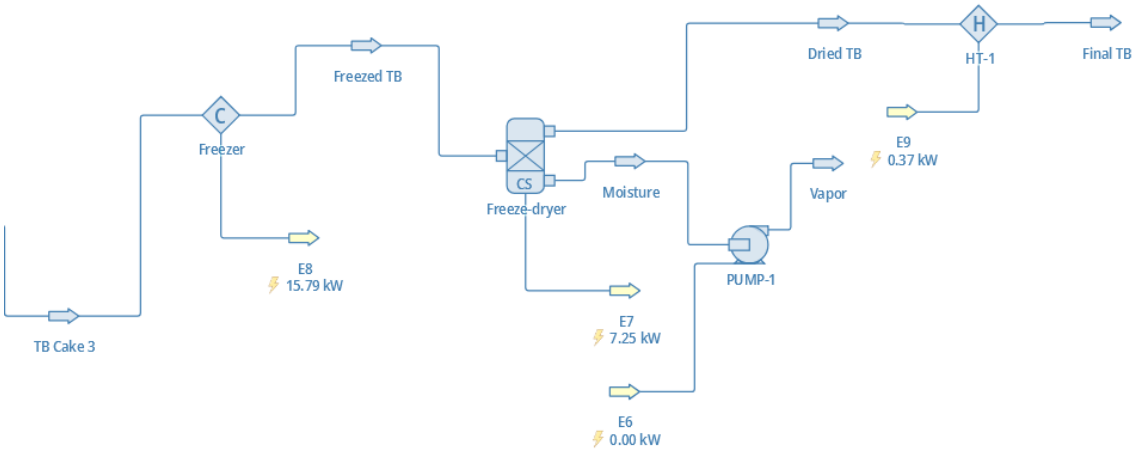
B



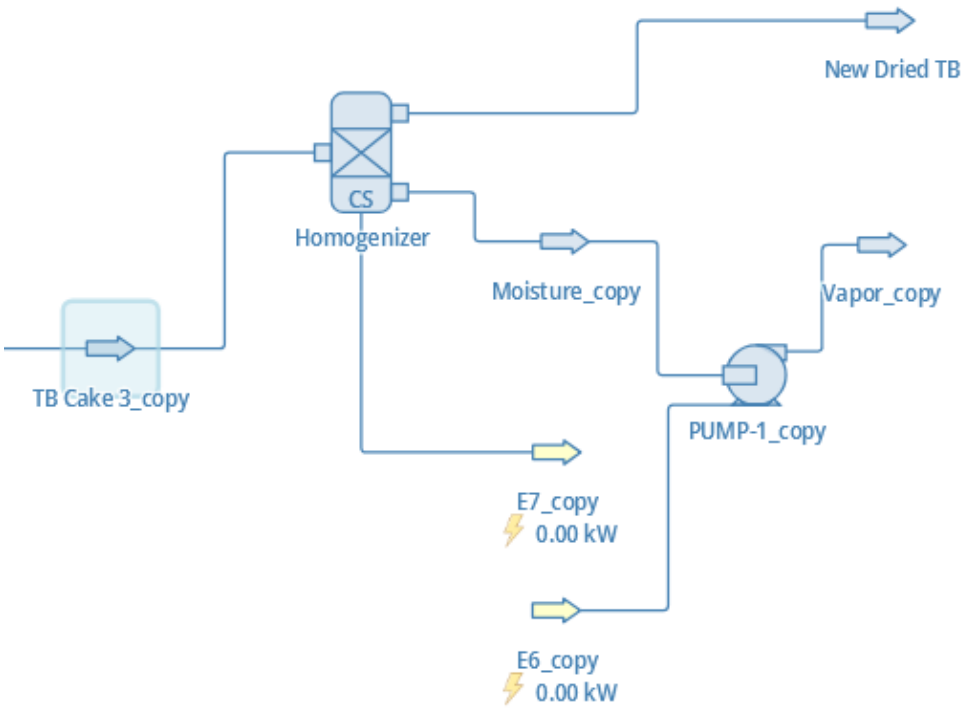
C


Cultivated Media
Centrifuge 1
Supernatant 1
TB Cake 1
E2
0.00 kW
Washing 1
Water 1
Washed TB 1
Centrifuge 2
Supernatant 2
TB Cake 2
E3
12.82 kW
Washing 2
Water 2
Washed TB 2
Centrifuge 3
Supernatant 3
TB Cake 3
E4
0.19 kW

D


TB Cake 3
Freezer
Freezed TB
E8
15.79 kW
Freeze-dryer
Moisture
Vapor
PUMP-1
E7
7.25 kW
E6
0.00 kW
Dried TB
HT-1
Final TB
E9
0.37 kW

E


TB Cake 3_copy
Homogenizer
New Dried TB
Moisture_copy
Vapor_copy
PUMP-1_copy
E7_copy
0.00 kW
E6_copy
0.00 kW

F

Supplementary Figure 1. We present the process flowsheet of TB production in DWSIM in Supplementary Figure 1A-F. A: Flowsheet for Pre-treatment in DWSIM.; B: Flowsheet for Inoculum Preparation in DWSIM.; C: Flowsheet for Cultivation in Bioreactor in DWSIM.; D: Flowsheet for Centrifugation and Washing steps in DWSIM; E: Flowsheet for Freeze-dryer in DWSIM.; F: Flowsheet for Homogenizer in DWSIM.

REFERENCES

1. Sreemahadevan, S.; Sivakumar, K. V.; Palanisamy, M. Evaluation of the Open Source Process Simulator DWSIM for Bioprocess Simulation. *Period. Polytech. Chem. Eng.* **2024**, *68* (2), 195–202. DOI: 10.3311/PPch.23166.