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AQUACULTURE AND AGRICULTURE BIOMASS SIDE STREAM PROTEINS AND BIOACTIVES FOR FEED, FITNESS AND HEALTH PROMOTING NUTRITIONAL SUPPLEMENTS	
Grant agreement:	790956
Duration:	April, 2018 – December, 2022
Coordinator:	NOFIMA AS, Norway

PROJECT-BASED LEARNING

Bioactivity assays with *S. cerevisiae* model cultures grown on processed fish side-streams

Small Research Project

'Physiological and kinetic studies'

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the BBI grant agreement No 790956

<table>
<tr><td>Project title</td><td colspan="3">Bioactivity assays with S. cerevisiae model cultures grown on processed fish side streams – physiological and kinetic studies</td></tr>
<tr><td>Responsible Organization(s)</td><td colspan="3">R&D Center Biointech Ltd</td></tr>
<tr><td>Project duration</td><td colspan="3">5 months</td></tr>
<tr><td rowspan="2">Research Effort Categories (%)</td><td>Basic</td><td>Applied</td><td>Developmental</td></tr>
<tr><td>10</td><td>90</td><td>-</td></tr>
<tr><td rowspan="2">Background data</td><td>Field of science</td><td>Knowledge area</td><td>Subject of investigation</td></tr>
<tr><td>Biology/Biotechnology</td><td>Microbial growth on fish side streams</td><td>Metabolic response and adaptation mechanisms</td></tr>
<tr><td>Summary</td><td colspan="3">This project is focused on appraisal of yeast growth on non-traditional carbon and energy sources based on fish by-products hydrolysates as an indicator of cellular adaptation. The bioactivity assays were performed with S. cerevisiae model batch cultures by measuring physiological and kinetic parameters. The physiological and kinetic investigations indicate that the growth of S. cerevisiae model strain is effective, and the tested substrates are low-cost alternatives for production of yeast biomass containing valuable metabolites.</td></tr>
<tr><td>Project objectives</td><td colspan="3">Study the physiological behaviour and kinetic parameters of S. cerevisiae grown on non-traditional carbon substrate - fish by-product hydrolysates.</td></tr>
<tr><td>Project Methods</td><td colspan="3">Along the project, the following methods and techniques will be exploited:
- Batch cultivation technique;
- Physiological and analytical control of the growth process:
o Cell viability assay;
o C and N concentrations determination;
o Kinetic parameters determination.</td></tr>
<tr><td colspan="4">Accomplishments under project objectives</td></tr>
<tr><td>Work performance
Batch cultivation of the model S. cerevisiae strain, physiological and analytical control</td><td colspan="3">Results:
- <u>Viability assessment</u>: The batch cultures of S. cerevisiae model strain were grown on three tested media containing fish hydrolysates at 1% concentration. After 18 h of cultivation, the cultures were examined for their viability microscopically. Their physiological state was monitored until mid-log growth phase, and the percentage of viable, budding and dead cells calculated (Fig. 1 A-C.). During the first 6 hours of the tested period of growth the percentage of viable cells was high regardless of the medium used, and varied within narrow limits (76-97 + 3-4%). The budding cells percentage was also high since the cultures were metabolically active. The dead cells represented a negligible percentage of the total (< 10 + 0.4%). These results indicate that the processed side-stream media support viable and physiologically active populations of the model strain.
- <u>Carbon and nitrogen utilization</u>: Data about the analytical control of the cultures showed enhanced glucose metabolism in the first 12 hours of cultivation when the cells grew at an exponential growth rate (Fig. 2 A-C). The glucose was catabolized, the cells were dividing rapidly, and the biomass concentration increased with maximum growth rate (μ^{max}). The amine nitrogen was also readily consumed by all cultures.
- <u>Kinetic parameters</u>: The values of the kinetic parameters: specific growth rate</td></tr>
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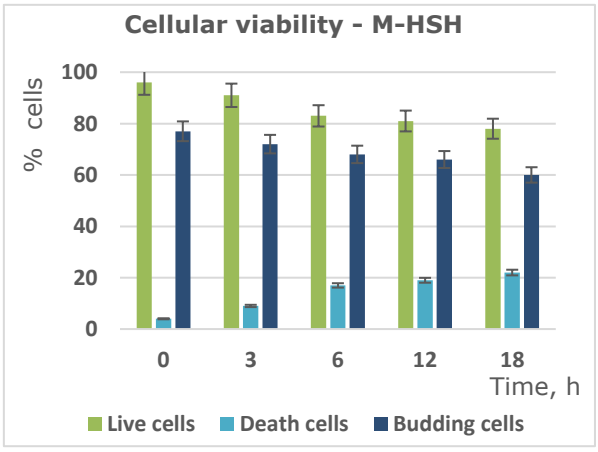
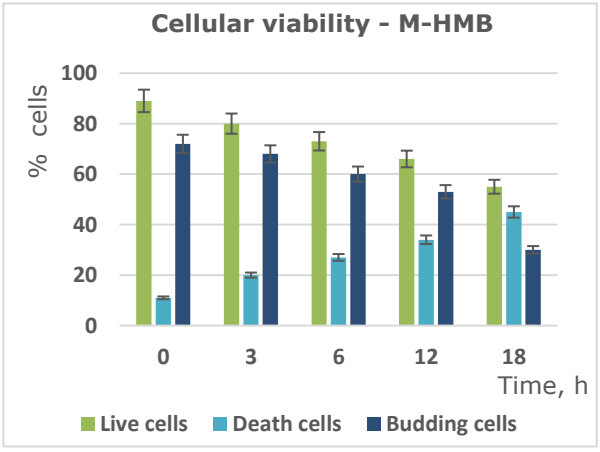
	(μ), growth yield coefficient (Yx/s), and the specific rate of substrate utilization (qs), are presented in Table 1. *S. cerevisiae* strain grew intensively with μ of 0.41 ± 0.01 h-1 - 0.46 ± 0.02 h-1 on the nutrient media with fish hydrolysates. The strain utilized the carbon source with good efficiency: Yx/s range of 0.37 + 0.03 - 0.43 + 0.02. High values for qs (0.28 + 0.01 – 0.31 + 0.02 h^{-1}) were registered. These results indicate the beneficial effect of the processed side streams on the growth and development of the yeast population. **Conclusions:** - The physiological and kinetic investigations indicate that the growth of *the S. cerevisiae* model strain on media containing 1% fish hydrolysates is effective. These organic substrates are a low-cost alternative for the production of yeast biomass containing valuable metabolites. It exhibited 98 + 2% viability and the best performance in terms of growth rate, biomass yield, specific growth rate, and substrate consumption during growth on fish hydrolysates. The results indicate that the model strain can carry out simultaneous respiration and fermentation at 28 °C. - The obtained results indicate active utilization of the fish hydrolysates that could be used as a principal advantage for innovation of the process of yeast cell factory expression.
References	1. Pirt S.J. (1975) Principles of microbe and cell cultivation, Oxford, Blackwell Scientific Publ., 4-44. 2. Miller G.L. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*. 31, 3, 426–428. 3. Taylor H. W. (1957) Formol titration: an evaluation of its various modifications. *Analysts*, 976, 488.

RESEARCH DATA:



A



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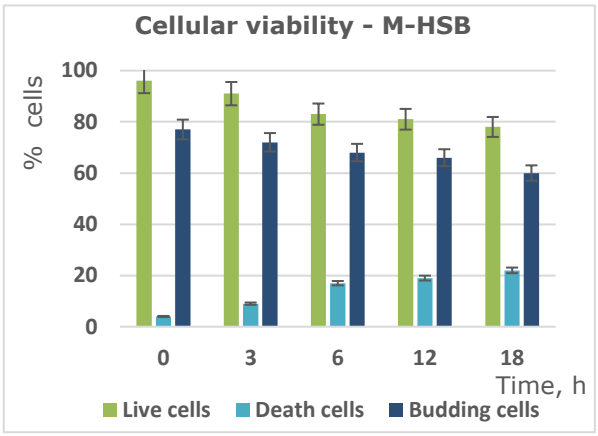
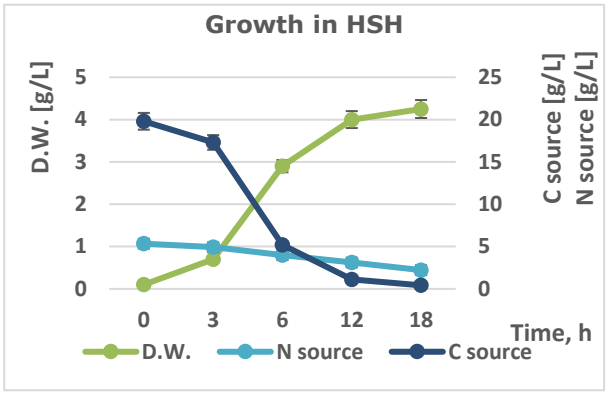
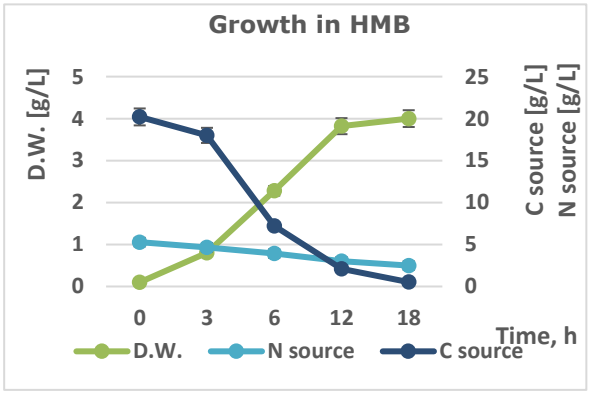


C

Figure 1. Cellular viability of *S. cerevisiae* strain during growth in different nutrient media containing fished hydrolysates: A) M-HSH, B) M-HMB and C) M-HSB; $p < 0.05$.



A



B

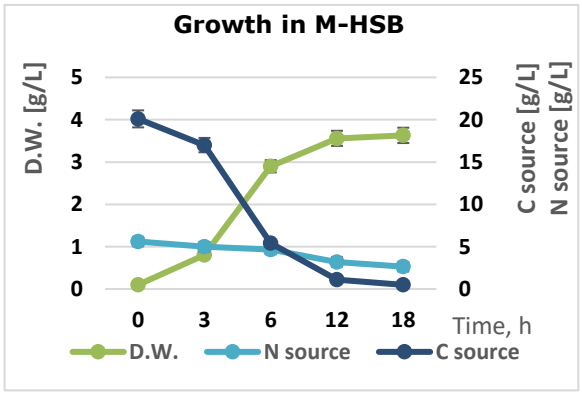


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Figure 2. Growth, carbon, and nitrogen utilization during batch cultivation of *S. cerevisiae* strain during growth in different nutrient media containing fished hydrolysates: A) M-HSH, B) M-HMB and C) M-HSB; $p < 0.05$.

Table 1. Kinetic parameters of the yeast culture and inhibitory/stimulatory effect.

Medium	μ (h^{-1})	$Y_{x/s}$	q_s (h^{-1})
M-HSH	0.46 ± 0.02	0.39 ± 0.01	0.29 ± 0.01
M-HSB	0.41 ± 0.01	0.37 ± 0.03	0.31 ± 0.02
M-HMB	0.43 ± 0.02	0.43 ± 0.01	0.28 ± 0.01

LEARNING BENEFITS:

<table>
<tr><td>Learning Outcomes</td><td colspan="3">After successful accomplishment of project activities, the trainees are able to:</td></tr>
<tr><td></td><td><u>Knowledge</u>
- Describe physiological and kinetic investigations with yeasts
- Match experimental data and theoretical considerations
- Define approaches for bioactivity assays arrangement</td><td><u>Skills</u>
- Apply microbiological and kinetic methods
- Demonstrate techniques for microbial bioactivity assays performance
- Use specialized equipment</td><td><u>Autonomy/responsibility</u>
- Collaborate with colleagues
- Carry out tasks independently
- Present and report research data</td></tr>
<tr><td colspan="4">Outputs / Impact</td></tr>
<tr><td>Target audience trained</td><td colspan="3">This project is foreseen for:
- Graduates and Postgraduates (MSc, PhD students)
- Post-doctoral Researchers and Research Associates
- Academic professionals (Tutors)</td></tr>
<tr><td>Research results disseminated to communities of interest</td><td colspan="3">Distribution of project results at intra-institutional and intra-partnership level</td></tr>
<tr><td>Opportunities for training and professional development</td><td colspan="3">The benefits for the project targets:
- New specific knowledge and skills acquired
- Problem/solution-based thinking mastered
- Team-working abilities enhanced
- Understanding of circular economy principles clarified</td></tr>
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