

ASSESSMENT OF THE SAFETY OF MICROALGAL BIOMASS GROWN ON EITHER NUTRIENT-RICH PROCESS WATER OR CARBON DIOXIDE SIDE-STREAMS: STEPS USED AND CAVEATS CONCERNING ALGAL UTILIZATION

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Introduction

Growth of microalgae on nutrient-rich waste or carbon dioxide (CO₂) side-streams generated from food or energy production is a promising and sustainable approach for biomass & ingredient generation. Microalgae for use in food and feed applications must comply with food and feed safety laws and the level of contaminants, allergens, or hazardous substances including pathogenic bacteria, viruses and fungi generated during microalgae recovery, collection and processing must be documented and within limits outlined by the European Food Safety Authority (EFSA) if products are for sale in the EU27. Five microalgae derived bioactive ingredients - astaxanthin, β-carotene, phycocyanin, omega-3 fatty acids (EPA and DHA), and selected algal biomass including Spirulina and Chlorella species are currently approved for use as food/feed ingredients in the EU27. However, research has demonstrated the potential of several other microalgae for use as foods, functional foods and feed ingredients. Herein, we assess a number of microalgae grown on side-streams for their microbiological safety, iodine content and heavy metal contamination.

Methods

The presence and quantity of pathogenic bacteria including Coagulase positive *Staphylococcus* sp., Coliforms, *Bacillus cereus* and *Salmonella* sp. were determined using developed SOPs based on ISO methods ISO 4833-1, ISO 6888-1, ISO 4832, AFNOR-AES/0/3-09/00, ISO 7932.2004 and ISO 6579.1.2017. Heavy metal content was determined according to a published method (Napan et al., 2015, Figure 1). The iodine content of the biomass was determined using the Iodine Colorimetric Assay Kit (BioVision, California, USA, Figure 2), according to the manufacturers' instructions.



Figure 1: Heavy metal analysis using chromatography

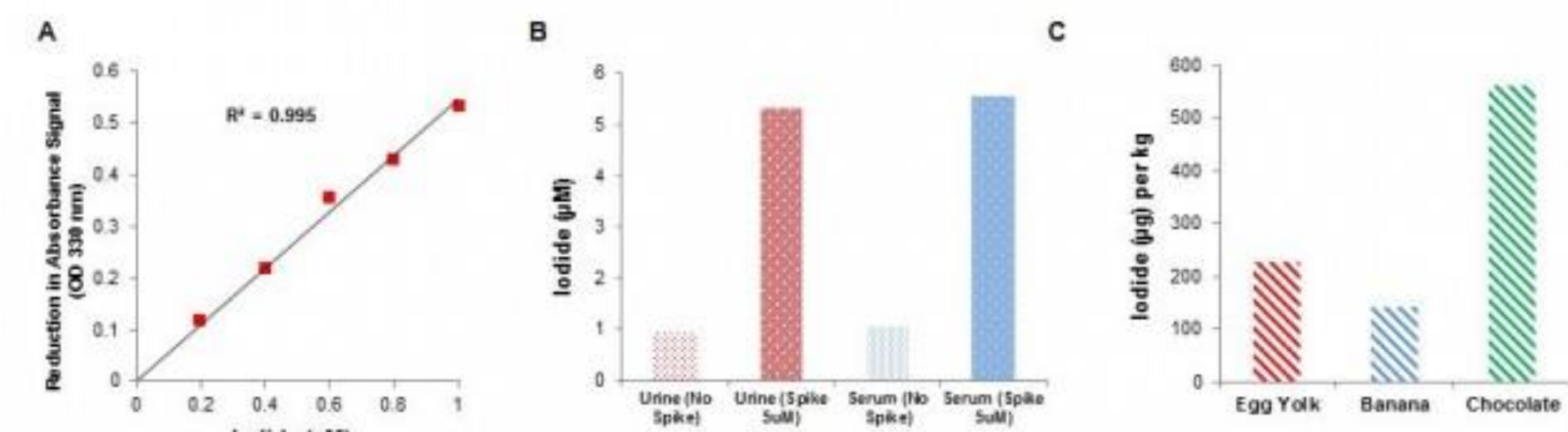


Figure 2: Iodide analysis using the Biovision assay method

Results & Conclusion

Microalga	Sidestream used for growth	Aerobic plate count ¹	Coagulase positive <i>Staphylococcus aureus</i> ²	Coliforms ³	<i>E. coli</i> ⁴	<i>L. innocua</i> ⁵	<i>B. cereus</i> ⁶	<i>Salmonella</i> sp. ⁷
<i>Nannochloropsis</i> sp.	MAF-permeate from open pond culture growing on demineralization process water. No filtration of permeate after storage.	2200 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	Not detected in 25 g	<20 CFU/g	Not detected in 25 g
<i>Defatted Nannochloropsis</i>	MAF-permeate from open pond culture growing on demineralization process water	1800 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	Not detected in 25 g	<10 CFU/g	Not detected in 25 g
<i>Chlorella</i> sp.	CO ₂ from biogas incineration	>3000000 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	Not detected in 25 g	<10 CFU/g	Not detected in 25 g
<i>Mix of algae</i>	Demineralization process water	>3000 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	Not detected in 25 g	<10 CFU/g	Not detected in 25 g
<i>Chloromonas</i> sp.	MAF-permeate from open pond culture growing on demineralization process water	830 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	Not detected in 25 g	<10 CFU/g	Not detected in 25 g

¹ Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique; ² Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) — Part 1: Method using Baird-Parker agar medium; ³ ISO 4832:2006
Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique; ⁴ ISO 7932:2004
Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive *Bacillus cereus* — Colony-count technique at 30 degrees C; ⁵ ISO 6579-1:2017
Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp.

Several microalgal cultures were found to contain excessive numbers of aerobic bacteria but with the exception of *Nannochloropsis* sp. none contained in excess of 10 CFU/g of the spore former *Bacillus cereus*. No samples contained excess iodine and heavy metal analysis is on-going.

