IMPROVING THE CIRCULARITY OF AQUACULTURE IN THE SOUTH BALTIC REGION – A PILOT STUDY ON THE USE OF MICROALGAE IN RAS SYSTEMS WITH WHITELEG SHRIMP *Penaeus vannamei*

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INTRODUCTION

Due to climatic conditions and the lack of permanent access to warm seawater, the production of whiteleg shrimp *Penaeus vannamei* (**Fig. 1**) in most of Europe is practically only feasible in land-based recirculating aquaculture systems (RAS). Although such systems allow for significant waste reduction by recycling and reusing water, they are not 100% efficient, especially at high stocking densities. Some of the water in the RAS must therefore be regularly replaced. Thus, there is ongoing interest in research focused on developing more effective circular economy solutions for RAS systems which aim not only to minimize waste but also to generate additional value. One promising approach is the culture of microalgae, which purify nutrient-rich effluent from a recirculating water system (RAS) during growth and, in addition, produce a biomass rich in omega-3 fatty acids that can serve as a dietary supplement for shrimp. The aim of our pilot study was to identify Baltic microalgae strains able to grow in wastewater from whiteleg shrimp RAS aquaculture, while producing a high yield of biomass.

Figure 1. Whiteleg shrimp *Penaeus vannamei* (photo credit: Piotr Kendzierski).

MATERIALS AND METHODS

STEP 1 & 2

A small-scale laboratory RAS system was developed, consisting of a main unit and four shrimp water tanks with a total capacity of 1200 L (**Fig. 2**).

P. vannamei post-larvae (n=1000) were imported from a hatchery in Austria (White Panther Produktion GmbH) and reared under controlled conditions: T=27°C, S=30 PSU, pH ~7.8, photoperiod 15 h L : 9 h D.

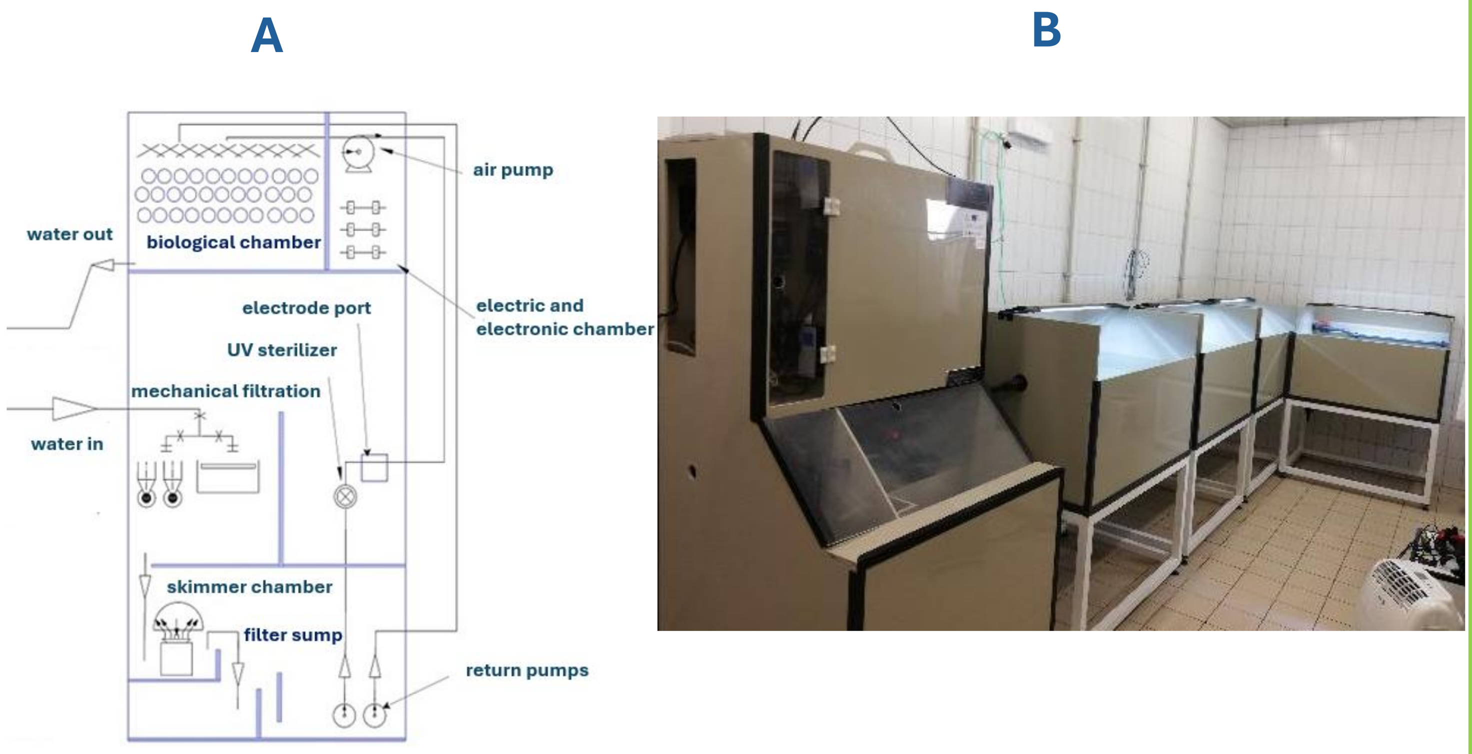

Figure 2. Small-scale laboratory RAS system: A - scheme of the main unit (AquaMedic Poland Bartosz Blum) and B – main unit (on the left) and four tanks (photo credit: Joanna Hegele-Drywa).

STEP 3

Ten liters of wastewater were collected from the RAS at week 13 of shrimp culture, and nutrient concentrations were determined using colorimetric cuvette tests (Hach Lange GmbH, Germany), which were: 1 mg L^{-1} for ammonium nitrogen, 122 mg L^{-1} for nitrate, 3 mg L^{-1} for phosphate, and 1 mg L^{-1} for silica. Three diatom strains were then selected for study: *Amphora* sp. CP3, *Gedaniella* sp. PL1.21, and *Nitzschia* cf. *aurariae* BA158, all from the University of Gdansk's own collection. Media for microalgae culture were prepared in eight variants (4 replicates for each one) using two salinities - details are given in **Table 1** (section Results).

Microalgae culture was started (**Fig. 3**) and the growth was monitored by measuring the optical density at 750 nm (converted later to cell density) every other day for 12 days (T = 20 °C, light intensity 100 µmol photons m^{-2} s^{-1}). The concentrations of photosynthetic pigments (chlorophyll a and fucoxanthin), the lipid concentration in the biomass and finally the concentrations of nutrients in all microalgal cultures were also determined.

Figure 3. Flasks with cultures of three diatom strains used in the studies (photo credit: Filip Pniewski).

RESULTS

All of the tested strains actively grew in the RAS wastewater (**Fig. 4A**), however the highest biomass (423 ± 10 mg L^{-1}) was observed for *N.* cf. *aurariae* BA158 cultured in the diluted RAS water supplemented with increased silica and iron amounts (variant 8, **Tab. 1**), which was comparable to the control culture (variant 1, **Tab. 1**).

Similarly, for *N.* cf. *aurariae* BA158 cultured in the diluted RAS water supplemented with increased silica and iron (variant 8, **Tab. 1**) the highest concentrations of lipids (69.5 mg L^{-1}, **Fig. 4B**), fucoxanthin (0.74 ± 10 mg L^{-1}, **Fig. 4C**) and chlorophyll a (1.95 ± 0.05 mg L^{-1}) were recorded.

All strains removed a significant portion of nutrients from wastewater (**Tab. 2**), but the most effective was *Amphora* sp., which removed as much as 85% of nitrates (variant RAS+BAL) and 80% of phosphates (variant RAS+BAL2.0).

Table 1. Characteristics of eight medium variants used for microalgae cultivation (ASW – artificial salt water).

No	Variant	Characteristics
1	f/2 30 PSU	f/2 medium prepared using ASW
2	f/2 18 PSU	f/2 medium prepared using ASW
3	RAS	Unchanged RAS water
4	RAS+BAL	RAS and Baltic water mixed in the ratio of 1:1 to reach a salinity of 18 PSU
5	RASm	RAS water with the increased P concentration to obtain N:P ratio of 24:1 (as in the f/2 medium)
6	RAS+BALm	RAS and Baltic water mixed in the ratio of 1:1 to reach a salinity of 18 PSU with the increased P concentration to obtain N:P ratio of 24:1 (as in the f/2 medium)
7	RASm2.0	RAS water with the addition of silica and microelements (including Fe)
8	RAS+BALm2.0	RAS and Baltic water mixed in the ratio of 1:1 to reach a salinity of 18 PSU with addition of silica and microelements (including Fe)

Table 2. Percentage of nitrates ($\Delta N\text{-}NO_3^-$) and phosphates (ΔP) removed by three diatom strains in six medium variants containing wastewater from RAS.

Variant	*Gedaniella* sp. PL1.21		*Amphora* sp. CP3		*Nitzschia* cf. *aurariae* BA158	
	$\Delta N\text{-}NO_3^-$	ΔP	$\Delta N\text{-}NO_3^-$	ΔP	$\Delta N\text{-}NO_3^-$	ΔP
RAS	34	66	77	3	10	10
RAS+BAL	69	31	**85**	51	56	51
RASm	52	48	77	34	24	37
RAS+BALm	61	39	77	59	53	56
RASm2.0	50	50	41	48	34	39
RAS+BALm2.0	68	32	57	**80**	45	70

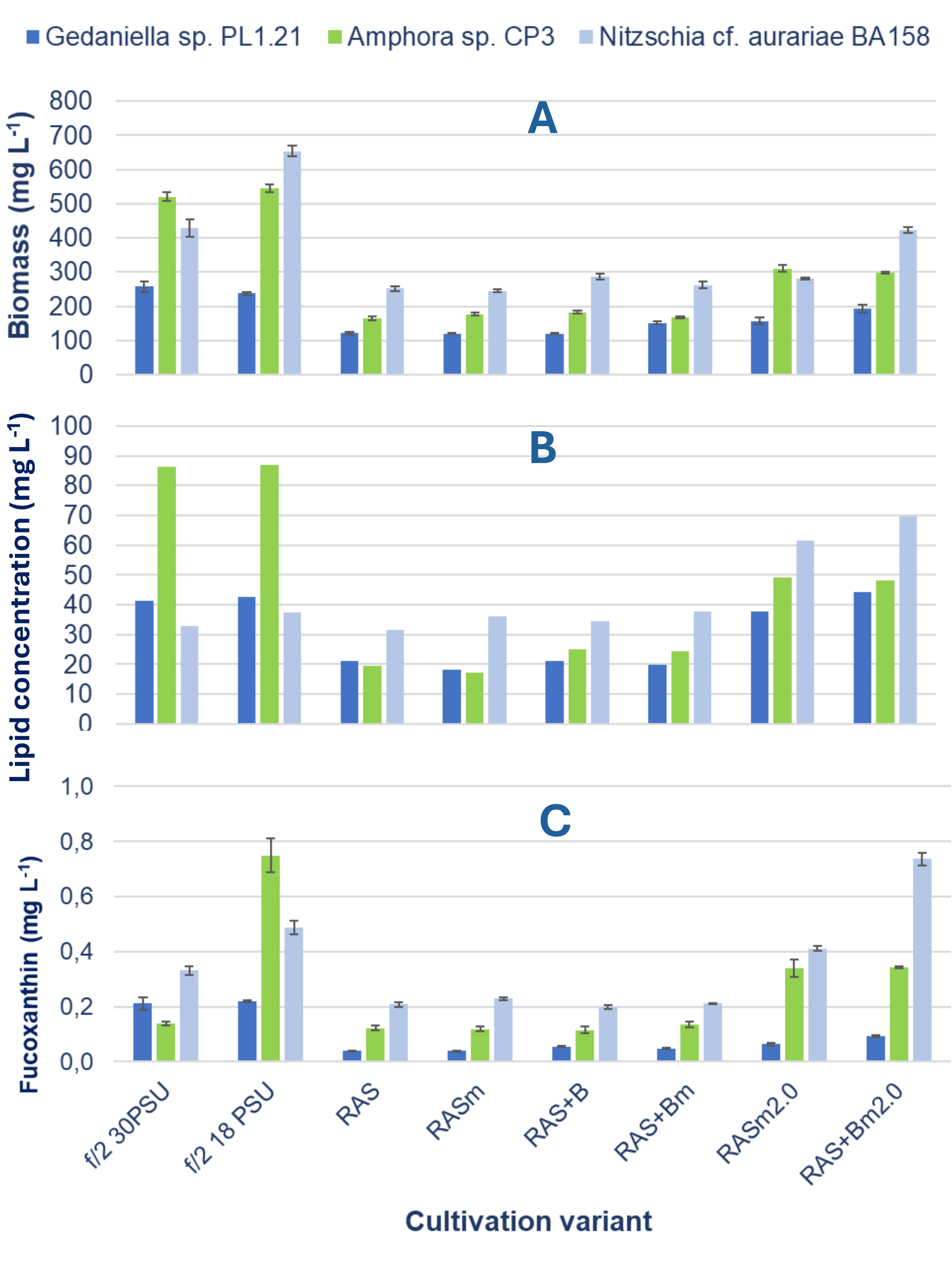

Figure 4. Mean (± SE) maximum biomass (A), mean lipid (B) and mean (± SE) fucoxanthin (C) concentrations of three diatom strains in all medium variants.

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CONCLUSIONS

The results of this pilot study showed that the RAS wastewater can be used as a medium for the efficient growth of Baltic Sea microalgae to remove excess nutrient concentrations. However, the success of this approach depends on appropriate modification of the wastewater to achieve the correct N:P ratio and, if necessary, to adjust silica and iron concentrations. Since all of the strains efficiently removed nutrients, the choice of the strain for cultivation should be based on its ecophysiological and biochemical traits to produce high-quality and commercially viable biomass, thereby adding value to RAS systems.