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MIXOTROPHIC CULTIVATION OF MICROALGAE IN WHEY MEDIUM FOR POULTRY DIET SUPPLEMENTATION WITH MICROALGAL BIOMASS

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Abstract: During this research microalgal strains of *Graesiella emersonii* KM01 and *Tetradesmus obliquus* OM02 were isolated from various freshwater bodies, also microalgal cultures *Chlorella vulgaris* CCAP 211/111, *Scenedesmus quadricauda* CCAP 276/16, *Chromochloris zofingiensis* CCAP 211/14 were from Culture Collection of Algae and Protozoa (CCAP, United Kingdom) and *Galdieria sulphuraria* UTEX 2919 from Culture Collection of Algae of the University of Texas at Austin (UTEX, USA). It has been shown that to increase microalgae biomass yield, it is possible to cultivate microalgae mixotrophically in whey medium. By comparing growth performance of microalgal cultures in lactose containing media and whey, a promising approach for achieving high biomass yield, thus also for decreasing environmental impact of improper whey disposal, was demonstrated for *T. obliquus* OM02. Growth of both *G. emersonii* KM01 and *C. zofiengiensis* CCAP 211/14 on lactose containing media and whey was found to be relatively low, not exceeding biomass productivity of isolate *T. obliquus* OM02. Cultivation of *T. obliquus* OM02 in whey demonstrated that it is possible to achieve the highest biomass in 50 % diluted whey, which resulted in 2.83 ± 0.11 g/L and is the highest yield between microalgal isolates in this research. Results confirm that mixotrophic cultivation of certain lactose assimilating microalgal isolates in whey compared to photoautotrophic cultivation enables to obtain a comparable biomass yield, which can be attributed to whey medium composition.

Key words: microalgae, *Graesiella emersonii, Tetradesmus obliquus,* whey, mixotrophic cultivation

Introduction

As the global human population is increasing every day, also the demand for goods follows. To be able to deliver enough goods for human consumption, scientists need to expand research not only on land, but also on freshwater and saltwater bodies. There is a group of aquatic microorganisms that is drawing favorable attention by scientists – microalgae (Cheirsilp et al., 2023).

Poultry farming has a great impact on delivering human diet with eggs and meat. To obtain eggs and meat of high quality, an adequate diet should be provided for poultry being rich in lipids, polyunsaturated fatty acids, proteins, vitamins, complex carbohydrates, and other functional compounds (Uguz & Sozcu, 2023).

Microalgae are microorganisms that can be used to fulfil those requirements. It has been demonstrated that, to increase microalgae biomass yield, it is possible to cultivate microalgae mixotrophically in a media supplemented with industrial and agricultural by products (Abril Bonett et al., 2020; Chong et al., 2022; Khanra et al., 2021; Mat Aron et al., 2021; Vidya et al., 2023). By use of whey for microalgal cultivation media, it is attainable to decrease ecological footprint of its improper disposals, as it is known that whey considerably composes most important part of dairy processing by-products, as well as reduce production cost beside increased biomass production (Pescuma et al., 2015). However, only certain microalgal cultures are able to consume lactose, which is the main sugar component in whey. So, search of microalgal strains which are able to consume lactose as carbon source during mixotrophic cultivation is required (Doebbe et al., 2007).

This study aimed to evaluate the possibility for use of lactose assimilating freshwater microalgal isolates in whey mixotrophic bioconversion as industrial by-product and potential source for efficient microalgal biomass production. In respect of that, the tasks of the present research were as follows:

- to evaluate heterotrophic growth of microalgal cultures in carbohydrates (lactose, glucose, galactose) containing media;
- to evaluate biomass production of chosen microalgal isolates in whey or whey media.

Material and methods

Microalgal cultures

Freshwater microalgal strains of *Graesiella emersonii* KM01, *Tetradesmus obliquus* OM02 and unidentified strain X1 isolated from different water bodies in Riga (Latvia), were obtained from Microalgae Culture Collection of the Institute of Biology (University of Latvia). Microalgal strains of *Chlorella vulgaris* CCAP 211/111, *Scenedesmus quadricauda* CCAP 276/16, *Chromochloris zofingiensis* CCAP 211/14 were obtained from Culture Collection of Algae and Protozoa (CCAP, United Kingdom); *Galdieria sulphuraria* UTEX 2919 culture was obtained from The Culture Collection of Algae of the University of Texas at Austin (UTEX, USA).

Media and cultivation conditions

In this study microalgae were cultivated in Erlenmeyer flasks. Media for heterotrophic cultivation (3N-BBM-V) were supplemented with sugars (lactose, glucose, or galactose) in 5 g/L concentration. For mixotrophic experiments whey from AS "Rankas piens" was used, diluted with distilled water to obtain needed concentrations. For control in all experiments the 3N-BBM-V medium without added sugars was used. The mixotrophic cultivation was carried out in presence of LED light source with day: night cycle 16 : 8 h and light intensity of 80 μmol/m^{2}s. Heterotrophic cultivation was carried out in dark. Both cultivations were performed statically in an incubator at 25 °C for 14 days.

Biomass acquisition

After cultivation, samples of 50 mL culture liquids were collected, centrifuged at 8000 rpm for 5 minutes, then supernatant was discarded, and biomass resuspended in distilled water to eliminate media residues. Further, rinsed microalgal biomass has been transferred into pre-weighted weighting bottles and dried at 80 °C for 24 h.

Statistical analysis

All experiments performed in four replications ($n = 4$). One-way analysis of variance (ANOVA) was performed using SPSS (BM SPSS Statistics for Windows, Version 21.0; IBM Corp, Armonk, USA) to compare means at a significance level $p = 0.05$.

Results and discussion

In a course of this research obtained environmental isolates and microalgal cultures from culture collections were used for screening of lactose assimilation. Obtained isolates have been identified as *Graesiella emersonii* KM01 and *Tetradesmus obliquus* OM02, as well as unidentified isolate X1. Growth of isolates has been compared to that of *Chlorella vulgaris* CCAP 211/11, already being commercially widely used, and other cultures – *Scenedesmus quadricauda* CCAP 276/16, *Chromochloris zofingiensis* CCAP 211/14, *Galdieria sulphuraria* UTEX 2919. Heterotrophic growth pattern demonstrates (Figure 1), that all the used microalgal cultures prefer glucose, as it is monosaccharide and is more accessible to microalgae metabolism. Heterotrophic growth of *G. emersonii* KM01 and *T. obliquus* OM02 and *C. zofingiensis* CCAP 211/14 on lactose showed high biomass yield, compared to other cultures, respectively 0.20 ± 0.03 g/L, 0.08 ± 0,01 g/L and 0.29 ± 0,01 g/L, respectively, which could be related to higher *lacZ* gene activity. This gene is responsible for β – galactosidase enzyme production in microalgae and further lactose hydrolysis into monomers. Other microalgal cultures are less active in using lactose, which could be attributed to a lower β – galactosidase production by the strains (Bentahar et al., 2019). By comparing results of *G. emersonii* KM01*, T. obliquus* OM02 and *C. zofingiensis* CCAP 211/14 to other strains, all of them demonstrated significantly ($p < 0,05$) higher biomass yield compared to other as well as significant differences between the strains. Overall, there are not sufficient research made to investigate β – galactosidase activity in microalgae strains. In research carried out by (Zanette et al., 2019), eight microalgae strains were cultivated, from which only three demonstrated β – galactosidase activity in growth media supplemented with lactose at concentration 5 g/L.

By performing further mixotrophic growth experiments in whey, it has been assessed that both environmental isolates and *C. zofingiensis* CCAP 211/14 demonstrated higher biomass productivity than in photoautotrophic control, which can be attributed to the fact that during mixotrophic growth both light and organic carbon sources for microalgae are available for biomass synthesis and metabolic reactions. It means, that more overall energy is available to be diverted to biomass and metabolites production, than just for cell maintenance processes (Smith et al., 2015).

Results demonstrate that for *G. emersonii* KM01 biomass production the optimal whey concentration is 20 % (Figure 2). At this whey concentration it is possible to obtain 1.93 ± 0.06 g/L of dry biomass, which is by 89.12 % more than in control group grown in the 3N-BBM-V medium without whey supplement. Lowest results were obtained from 100 % and 5 % whey concentrations in the medium, 1.09 ± 0.05 g/L and 1.07 ± 0.04 g/L, respectively. It is about 56 % less, than it has been possibly to acquire from medium supplemented with 20 % whey.

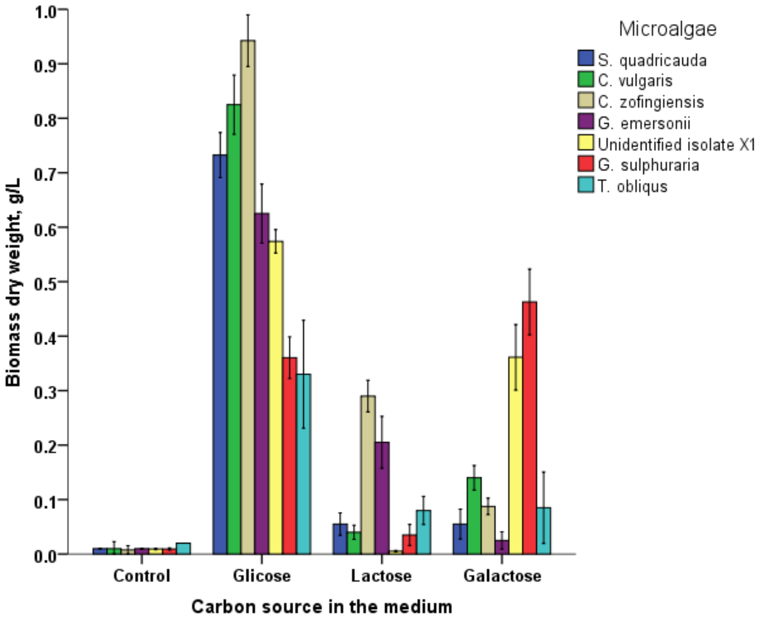

Figure 1. Heterotrophic growth of *Scenedesmus quadricauda* CCAP 276/16, *Chlorella vulgaris* CCAP 211/11, *Chromochloris zofingiensis* CCAP 211/14, *Graesiella emersonii* KM01, unidentified isolate X1, *Galdieria sulphuraria* UTEX 2919 and *Tetradesmus obliquus* OM02 in 3N-BBM-V media with added glucose, lactose or galactose compared to 3N-BBM-V control without any added sugars.

Results of *T. obliquus* OM02 cultivation in whey medium (Figure 3) demonstrate that whey concentration wherein the highest biomass is achievable is 50 %, which resulted in 2.83 ± 0.11 g/L of biomass and is the highest yield between three microalgal isolates.

By comparison to the control group (0.09 ± 0.01 g/L), it is by 96.82 % greater dry biomass weight. Notably, that *T. obliquus* OM02 demonstrated its ability to grow in high concentrations of whey, reaching dry biomass weight of 1.88 ± 0.25 g/L in the 100 % (undiluted) whey medium. This means, that it has great adaptability to produce β – galactosidase in presence of lactose in high concentrations, which results in higher presence of lactose monomers in media for use in its metabolic processes. As demonstrated in the Figure 1, microalgae prefer monosaccharides as they can be more easily metabolised (Smith et al., 2015).

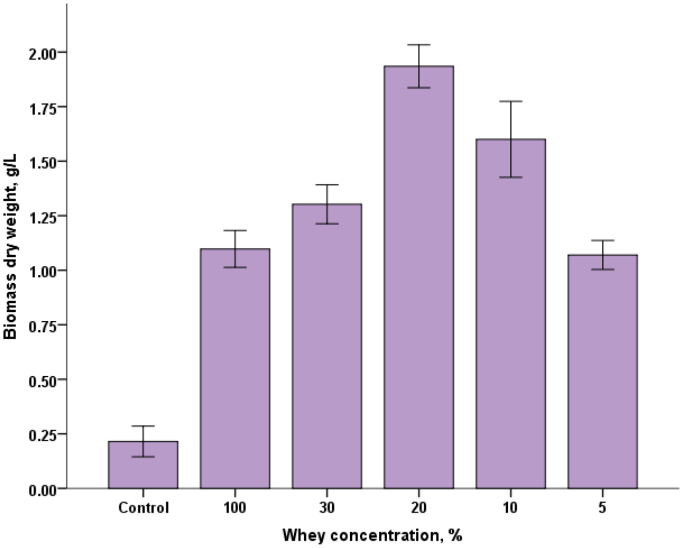

Figure 2. Graesiella emersonii KM01 growth in different whey concentrations (diluted with distilled water). Control – photoautotrophic cultivation in the 3N-BBM-V medium with no added whey.

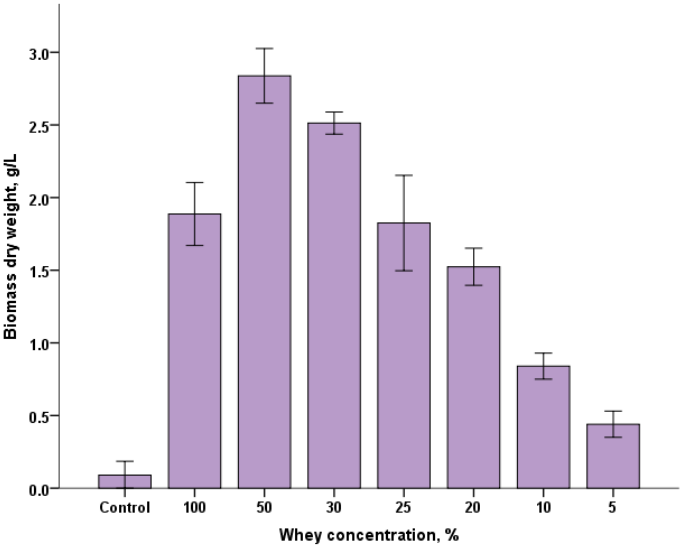

Figure 3. Tetradesmus obliquus OM02 growth in whey of different concentrations (diluted with distilled water). Control – photoautotrophic cultivation in the 3N-BBM-V medium with no added whey.

Results of *C. zofingiensis* CCAP 211/14 cultivation in whey medium (Figure 4) demonstrate that the optimal whey concentration is 6.5 %. By far this is the lowest optimal whey concentration between reviewed microalgal cultures. Dry biomass weight in the medium supplemented with 6.5 % whey was 0.72 ± 0.08 g/L. However, by comparison with control group (0.05 ± 0.005 g/L) it is by 93.06 % more. Whey concentration above 10 % showed low biomass production for this culture, it can be seen for 10 % diluted whey – 0.14 ± 0.01 g/L, for 20 % – 0.12 ± 0.01 g/L and for 100 % – 0.06 ± 0.01 g/L. Here clearly can be seen that high concentrations of lactose can cause inhibitory effect on biomass production (Fu et al., 2019).

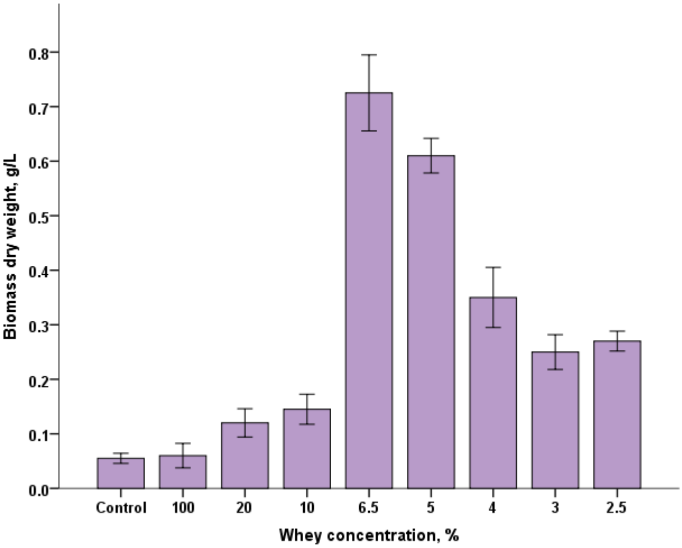

Figure 4. Chromochloris zofingiensis CCAP 211/14 growth in different whey concentrations diluted with distilled water. Control – photoautotrophic cultivation in the 3N-BBM-V medium with no added whey.

By comparing results of the three microalgae strains, the most promising one for achieving high biomass yield, thus also for decreasing environmental impact of improper whey disposal, was demonstrated to be *T. obliquus* OM02. Both *G. emersonii* KM01 and *C. zofiengiensis* CCAP 211/14 can be considered as relatively slow growing microalgae on lactose substrates, which do not exceed results of *T. obliquus* OM02. Results confirm that during mixotrophic growth in whey media it is possible to obtain higher biomass yield compared to photoautotrophic cultivation, which can be attributed to whey composition. Whey composition is rich in sugars, mainly lactose, also glucose, galactose, and traces of other sugars are present and can be potentially used by microalgae as an additional carbon source. It is also rich with minerals and other growth promoting factors, which could contribute to higher biomass yield. As well C/N ratio in whey could be more favorable then it is in a synthetic medium (Gao et al., 2019; Nabizadeh et al., 2020).

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