



Master thesis report

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Control mechanism and recommendations for eliminating a predatory contaminant in *Chlorella* cultures

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For a sustainable and European value chain of PHA-based materials for high-volume consumer products



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Title of Thesis: Control mechanism and recommendations for eliminating a predatory contaminant in *Chlorella* cultures

Summary (in English)

Extensive research spanning several years has focused on exploring the potential of *Chlorella* as a large-scale biomass source for various applications, including food, fertilizers, pharmaceuticals, chemical compounds, bioremediation, and energy production. However, a significant challenge arises from the susceptibility of *Chlorella* cells to being consumed by predators, resulting in decreased productivity. One particularly destructive predator in commercial *Chlorella* cultures is *Poteroiochromonas malhamensis*, a mixotrophic flagellate known for rapidly causing the collapse of microalgal mass cultivation within a short period. Consequently, there is an urgent need for effective and safe methods to control and prevent contamination by the predatory organism. In this study, we explored the cultivation of *Chlorella vulgaris* in an ammonium nitrogen (AN) medium under alkaline conditions as a means to control *P. malhamensis* in laboratory conditions. Ammonium bicarbonate (NH_4HCO_3) was used as the source of AN. Our results demonstrated that NH_4HCO_3 concentrations corresponding to AN concentration of 120, 240, and 360 mg/L effectively controlled *P. malhamensis*, resulting in a remarkable 95% mortality within 24 hours. Furthermore, the NH_4HCO_3 concentration corresponding to a AN concentration of 240 mg/L exhibited the highest biomass concentration, comparable to that of the control group conducted in nitrate medium. To validate the effectiveness of the method, co-cultures of *C. vulgaris* and *P. malhamensis* was conducted using an AN concentration of 240 mg/L. The biomass yield of *C. vulgaris* in treated co-cultures with AN was similar to that of the monocultures conducted in nitrate medium, while the untreated co-cultures experienced a contamination event and complete crash. Considering the AN toxicity to *P. malhamensis*, the relative safety to *C. vulgaris*, and its low cost and chemical safety, cultivation of *Chlorella* in NH_4HCO_3 under alkaline pH conditions emerges as a technically and economically feasible, as well as safe approach for controlling *P. malhamensis* contamination in outdoor, large-scale cultivation of *Chlorella*.

Keywords:

Poteroiochromonas malhamensis, *Chlorella vulgaris*, NH_4HCO_3 , NH_3 , ammonium nitrogen, contamination control

Summary (in Estonian)

Põhjalik uurimistöö mitme aasta vältel on keskendunud *Chlorella* potentsiaali uurimisele suuremahulise biomassi allikana erinevate rakenduste jaoks, sealhulgas toidu, väetiste, ravimite, keemiliste ühendite, bioremediatsiooni ja energiatootmise jaoks. Siiski esineb oluline väljakutse seoses *Chlorella* rakkude vastuvõtlikkusega röövloomade poolt söömisele, mis viib tootlikkuse vähenemiseni. Üks eriti hävitav röövloom kaubanduslikes *Chlorella* kultuurides on *Poteroiochromonas malhamensis*, segatoiduline lipikute liik, mille tuntud kiiresti põhjustada mikrovetika massikasvatuse kokkuvarisemist lühikese aja jooksul. Seetõttu on hädavajalik leida tõhusad ja ohutud meetodid röövloomade kontaminatsioon kontrollimiseks ja ennetamiseks. Käesolevas uuringus uurisime *Chlorella* vulgarise kasvatamist ammooniumlämmastikku (AN) sisaldavas keskkonnas leeliselises keskkonnas, et kontrollida *P. malhamensis*'t laboritingimustes. AN allikana kasutati ammooniumvesinikkarbonaati (NH_4HCO_3). Meie tulemused näitasid, et NH_4HCO_3 kontsentratsioonid vastavalt AN kontsentratsioonile 120, 240 ja 360 mg/L kontrollisid tõhusalt *P. malhamensis*'t, põhjustades märkimisväärse 95% suremuse 24 tunni jooksul. Lisaks näitas NH_4HCO_3 kontsentratsioon vastavalt AN kontsentratsioonile 240 mg/L kõrgeimat biomassisagedust, võrreldavat nitraadi keskkonnas läbiviidud kontrollrühmaga. Meetodi tõhususe kontrollimiseks viidi läbi *C. vulgaris* ja *P. malhamensis*'i ko-kultiveerimine AN kontsentratsiooniga 240 mg/L. *C. vulgaris* biomassitootlus töödeldud ko-kultuurides AN-ga oli sarnane nitraadi keskkonnas läbiviidud monokultuuridega, samas kui töötlemata ko-kultuurid kogesid saastumisjuhtumi ja täieliku kokkuvarisemise. Arvestades AN toksilisust *P. malhamensis*'le, suhtelist ohutust *C. vulgaris*ele, madalat hinda ja keemilist ohutust, on *Chlorella* kasvatamine NH_4HCO_3 keskkonnas leeliselise pH tingimustes tehniliselt ja majanduslikult teostatav ning ohutu lähenemisviis *P. malhamensis*'i kontaminatsioon kontrollimiseks välitingimustes suuremahulises *Chlorella* kasvatuses.

Märksõnad:

Poteroiochromonas malhamensis, *Chlorella vulgaris*, NH_4HCO_3 , NH_3 , ammooniumlämmastik, kontaminatsioon kontroll