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Bioremediation of chromium contaminated water by diatoms with concomitant lipid accumulation for biofuel production.

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Running title: Combined chromium removal and lipid production in marine diatoms

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Abstract:

Hexavalent chromium compounds such as chromate and dichromate, commonly designated as Cr (VI) compounds, are widely used heavy metals in different industries and are considered highly toxic to most life forms. Unfortunately, they have become a major pollutant of groundwater and rivers around dichromate using industries. Bioremediation is widely used to decrease the amount of dichromate in wastewater but requires large amounts of precious fresh water. Here we tested two marine micro-algal species, *Phaeodactylum tricornutum* strain CCY0033 and *Navicula pelliculosa* strain CCMP543, for their ability of dichromate bioremediation and concomitantly producing lipids that can serve as biofuel. Dichromate tolerance of the strains was investigated under different growth conditions in order to obtain high biomass yields, high lipid accumulation and high dichromate removal from the medium. Both algal strains grew well and produced high biomass in media containing up to 1 mg of dichromate per liter. Variations in growth conditions revealed that dichromate removal from the medium correlated positively with biomass yield. Dichromate removal using living cells was in the same order of magnitude as with autoclaved dead cells or when using extracted extracellular polymeric substances (EPS). This suggests biosorption of dichromate to cell-associated polymeric substances as the major mechanism of the bioremediation process. For both strains, optimal dichromate removal and lipid production were achieved at a light intensity of 55 µmol $m^{-2}s^{-1}$ and at a sodium nitrate concentration of 3 mM. The optimal temperature for dichromate removal and lipid production was 23 °C for *P. tricornutum* and 27 °C for *N. pelliculosa*. Compared to *P. tricornutum* strain CCY0033, *N. pelliculosa* strain CCMP543 produced an overall higher lipid yield under these conditions.

Keywords: diatoms, chromium (VI), bioremediation, lipids

1. Introduction

Chromium exists in three major forms in nature: the uncharged metallic form (Cr), a trivalent form (Cr (III)) and a hexavalent form (Cr (VI)) (Greenwood and Earnshaw, 1997). Chromium has several important industrial applications such as in the leather industry, chrome plating, textile manufacturing, and steel industry (U.S. Department of Health and Human Services, 2008). Cr and Cr (III) are considered nontoxic and non-carcinogenic or possess only low toxicity. Moreover, in trace concentrations, Cr (III) is even an essential element for life (Straif et

al., 2009; U.S. Department of Health and Human Services, 2016). Cr (VI), however, is classified as a human carcinogen (Straif et al., 2009) and is toxic to animals (Velma et al., 2009), plants (Shanker et al., 2005) and microorganisms (Agency for Toxic Substances and Disease Registry, 2012; Petrilli and De Flora, 1977; Wong and Trevors, 1988; Yao et al., 2008). An estimated 50 to 80 % of all plant and algal species are negatively affected by Cr concentrations exceeding 100 µg/L (Federal Environmental Quality Guidelines, 2017). Toxic concentrations of Cr (VI) for microalgae varied from 1 µg/L for the diatom *Thalassiosira pseudonana* to up to 10 mg/L for a *Chlorella* sp. (Wong and Trevors, 1988). Cr (VI) toxicity affects microorganisms in pure cultures (Petrilli and De Flora, 1977) as well as in natural microbial communities. Increasing the concentration of Cr (VI) resulted in decreased microbial activity (Yao et al., 2008).

Many ecosystems and surface- and groundwaters are polluted by heavy metals including Cr (VI), mostly in the form of chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) (Agency for Toxic Substances and Disease Registry, 2012), in which Cr (VI) concentrations range from 0.5 mg/L to up to 20 mg/L (Agency for Toxic Substances and Disease Registry, 2012; Zhitkovich, 2011). In 1991, the United States Environmental Protection Agency (US-EPA) has set the maximum allowable contamination level for total chromium at 100 ppb (100 µg/L). In a 2014 revision, the acceptable contamination level was decreased to 10 ppb (10 µg/L) and is aiming at a public health goal to reach 0.02 ppb (20 ng/L) (Federal Environmental Quality Guidelines, 2017; Agency for Toxic Substances and Disease Registry, 2012; U.S. Department of Health and Human Services, 2016). This has led to the development of various abiotic and biological approaches for the treatment of wastewater or ecosystems in order to eliminate Cr (IV) contamination (Barrera-Díaz et al., 2012). Bioremediation uses autochthonous or introduced living organisms, often microorganisms, algae, or plants in order to remove or detoxify contaminants. While plants, heterotrophic bacteria, and fungi have been widely used in bioremediation of Cr (VI) contaminated sites, the potential of applying phototrophic microorganisms has received much less attention (Barrera-Díaz et al., 2012). This is surprising because just as plants, microalgae and cyanobacteria are photoautotrophic and harvest sunlight energy, which would decrease process costs and could therefore be commercially attractive. In addition, microalgae and some cyanobacteria are known to accumulate lipids and hence their biomass can be used for the development of so-called third-generation biofuels. These organisms, in addition to their bioremediation capacities, can be grown on non-arable land and when choosing marine or salt-adapted organisms, competition with food production and the use of precious freshwater could be avoided (Sharma et al., 2012). Furthermore, biosorption using extracellular polymeric

substances (EPS) is one of the major mechanisms of heavy metal bioremediation by microalgae and cyanobacteria, and it would therefore be possible to recover metals such as Cr (IV) from these biosorbents (Sen and Dastidar, 2010).

Bacillariophyta (diatoms) comprise a large and diverse group of microalgae that are widespread in aquatic ecosystems. They are often the dominant group of eukaryotic phytoplankton and are responsible for 40-45% of the primary production in the ocean (Sarthou et al., 2005; Smetacek, 1999). Moreover, diatoms are key to a worldwide algae-based bioeconomy that is used for food, feedstock, and biofilm production (Laurens et al., 2017). There have been many applications using diatoms including bioremediation of heavy metals (Bozarth et al., 2009; Pereira et al., 2011). Moreover, several strains of diatoms are known to possess natural high contents of neutral lipids that can be converted into biodiesel (Zhu et al., 2016).

This study aimed at the use of diatoms to combine the sequestration of toxic chromium (VI) and the optimization of lipid accumulation for biofuel production. For this, we have used two species, *Phaeodactylum tricornutum* and *Navicula pelliculosa*. These benthic diatoms have been shown to be oleaginous (produce lipid) and produce high amounts of extracellular polymeric substances (EPS) while growing at low silicate concentration (Coombs et al., 1967; Kaur, 2014; Lewin, 1955). By varying the culturing parameters, we optimized growth yield, chromium removal, and lipid production.

2. Materials and methods

2.1 Organisms and culture conditions

The diatoms, *P. tricornutum* CCY0033, isolated from an intertidal sediment from the North Sea beach of the Dutch barrier island Schiermonnikoog, the Netherlands, and *N. pelliculosa* CCMP543/CCY0399, originally isolated from Oyster Pond, Martha's Vineyard, Massachusetts USA, were obtained from the Culture Collection Yerseke (CCY), Royal Netherlands Institute of Sea Research. Cultures were grown in MDV medium (Supplementary material) containing 6 mM nitrate and 150 µM silicate in 250 mL polystyrene tissue culture flask (TTP, 90026, Switzerland). To 92 mL of fresh medium, 8 mL (8% v/v) of an actively growing, 3 weeks old pre-culture was added. The cultures were incubated at 14 °C and 55 µmol $m^{-2}s^{-1}$ light (photon flux density) at a 16-8 h light-dark regime. The pH of the media was adjusted at 7±0.2. These conditions are hereafter called “standard growth conditions”. Culturing was

always performed in triplicate and the growth was followed by measuring the optical density at 600 nm (OD_{600}) using sterile MDV medium as a blank.

2.2 Chromium (VI) tolerance

To determine Cr (VI) tolerance, both diatoms were grown as described above in MDV medium containing Cr (VI) in the form of potassium dichromate $K_2Cr_2O_7$ at final concentrations ranging from 0 to 10 mg/L. Growth was assessed by end-point measuring the optical density at 600 nm (OD_{600}) after 15 days of incubation.

2.3 Analysis of dichromate concentration

Residual Cr (VI) in the medium after biosorption was assayed using a colorimetric test upon reaction with diphenyl carbazide (DPC) according to recommendations by the American Public Health Association (APHA Method 3500-Cr: Standard Methods for the Examination of Water and Wastewater (chromium), 1989 and 1996) and the microalgae samples were prepared as described by Dönmez and Aksu (2002). The samples were centrifuged for 15 min at 5,000 rcf before adding DPC to the supernatant. Hexavalent chromium concentration was determined by measuring the colorimetric at 540 nm using various concentration of Cr (VI) (0, 0.25, 0.5, 0.75, and 1 mg/L) in sterile MDV medium as calibration curve.

2.4 Evaluation of heat-killed P. tricornutum *and* N. pelliculosa *for chromium removal*

To establish potential modes of chromium removal (enzymatic versus passive biosorption), chromium removal by living cells were compared with removal with heat-killed diatom cells. Diatoms were grown for 15 days in Cr (VI)-free MDV medium (100 mL) and killed by autoclaving for 20 min at 121 °C. After cooling down, the 100 mL of heat-killed cells were transferred to a dialysis tube (3 kDa cut-off) and placed in a sterile flask (Blue Cap Screw Cap bottle, 500 mL, DURAN) containing 100 mL sterile MDV. Filter sterilized Cr (VI) was added to the medium at a final concentration of 1 mg/L and incubated under standard growth conditions while stirring with a magnetic bar at 150 rpm. Incubation was maintained for 3 days to establish an equilibrium in Cr (VI) concentration and the residual hexavalent chromium concentration in the external medium was determined as described above.

2.5 Potential role of extracellular polymeric substances in chromium (VI) biosorption

EPS is abundantly produced by both diatom species and can be present as colloidal molecules suspended in the medium or attached to the cells (Staats et al., 1999). Extraction of both EPS fractions was carried out according to Staats et al. (1999) with slight modifications. Non-attached EPS was separated from the cells in both diatom cultures (100 mL) by centrifugation at 20,000 rcf (15 min and 10°C). The loosely bound attached-EPS fraction was extracted from the cell pellet by resuspending it in 5 mL of sterile MDV medium and incubated for 1 h at 30 °C in a shaking water bath. Subsequently, the suspension was centrifuged at room temperature for 30 min at 20,000 rcf and the supernatant was combined with the non-attached EPS-containing supernatant from the previous step. Instead of precipitating the EPS, we followed Sharma et al. (2008) using EPS for heavy metal bioremediation. Briefly, the collected cell-free culture supernatant containing the EPS was filter sterilized (0.2 µm filters, cellulose acetate membrane, VMR, 514-0061), subsequently freeze-dried in a sterile bottle, and stored at -20 ºC until further use. For chromium biosorption testing, the freeze-dried EPS (extracted from 100 mL culture) was dissolved in 20 mL of sterile MilliQ water and put into a dialysis tube (3 kDa cut-off). The dialysis tube was placed in a flask containing 70 mL sterile MilliQ water then the volume was adjusted to 100 mL and filter sterilized Cr (VI) was added at a final concentration of 1 mg/L. The flasks with the EPS-containing dialysis tube were incubated under standard cultivation condition with mild shaking at 150 rpm. After 3 days the residual hexavalent chromium concentration in the medium was determined as described above.

2.6 Lipid measurement

The lipid accumulation in diatom cells was visualized using the fluorescent stain 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY 505/515; Invitrogen). The lipid content was measured using the sulfo-phospho-vanillin (SPV) assay (Mishra et al., 2014). Calibration was done with triolein (Sigma) as standard. The lipid concentration in the culture was expressed as µg triolein/mL.

2.7 Variation in growth condition of the diatoms

The effect of different growth conditions on biomass production, lipid production, and chromium bioremediation was investigated. These included different culture media, temperature, light intensity, nitrogen source and -concentration, silicate depletion, the presence of azide, and long vs short-term exposure to Cr (VI).

2.8 Statistics

The results were statistically analyzed using a one way ANOVA and testing the significance of the variance using Tukey's Honest Significant Difference (TukeyHSD). Both ANOVA and TukeyHSD were performed using the core R code (R Core Team, 2017). All experiments were performed in triplicate and p-values < 0.05 were considered significant.

3. Results and discussion

Different growth conditions were applied in order to test whether the diatoms *P. tricornutum* and *N. pelliculosa* are potential candidates for bioremediation and concomitantly accumulate high amounts of neutral lipids, which could be used as a resource for biofuels. For efficient Cr (VI) removal and obtaining high lipid yields, it is essential to maintain a relatively high biomass and only cultures with an OD600 > 0.2 were considered for further analysis.

For establishing the optimal condition for the aforementioned goals, several growth factors were tested in media containing 1 mg/L Cr (VI). The diatoms were grown in the presence of 1 mg/L of Cr (VI), in different artificial seawater media (MDV, ASNIII, and T+), different temperatures (14, 18, 23, 27, 30, and 37 °C), different light intensities (30, 55, and 80 µmol $m^{-2}s^{-1}$), different nitrogen sources (nitrate, nitrite, urea, and ammonium nitrate) at different concentrations (0, 0.6, 3, 4.5, 6 and 7.5 mM), and silicate concentration (1.5 and 150 µM). Furthermore, the cultures were treated by the respiratory inhibitor, sodium azide, at 0, 10, 25, 50, 100 µM. The effect of long- or short-term exposure to Cr (VI) was tested in cultures that were grown for 15 days in the presence of 1 mg/L Cr (VI) and cultures that were grown without Cr (VI) and subsequently exposed for 24 h to 1 mg/L Cr (VI).

3.1 Tolerance of P. tricornutum *and* N. pelliculosa *to Cr (VI) and their potential to remove it*

The diatoms revealed tolerance to Cr (VI) up to 1 mg/mL (Table 1). At concentrations of 5 and 10 mg/L the growth yield was considerably lower, even though *N. pelliculosa* appeared to be less susceptible. The biomass-normalized Cr (VI) removal was highest at 1 mg/mL. Both strains removed up to 30-35% of the added Cr (VI) (Fig. 1A & B). At 5 mg Cr (VI)/L, *N. pelliculosa* removed 20% albeit at a lower standing stock of biomass. At higher Cr (VI) concentrations the biomass was too low to obtain a significant Cr removal (Table 1). Hence, for application of the tested diatom strains for treatment of contaminated water the concentration of Cr (VI) should not exceed 1mg/L. Subsequent experiments were therefore performed at 1 mg/L.

In general, hexavalent chromium is considered toxic and carcinogen. For human purposes (e.g. drinking water) the maximum allowable concentration has been set at 10 ppb (10µg/L) and EPA aims to decrease it even further to less than 0.02 ppb (20 ng/L) for public health (Federal Environmental Quality Guidelines, 2017; Agency for Toxic Substances and Disease Registry, 2012; U.S. Department of Health and Human Services, 2016). Moreover, Cr (VI) has been shown to be highly toxic and non-essential for microalgae and bacteria, although some strains have shown some level of resistance to Cr (VI) (Agency for Toxic Substances and Disease Registry, 2012; Cervantes et al., 2001; Wong and Trevors, 1988). The toxic concentration depends on the species and ranges from 1 µg/L for the estuarine diatom *Thalassiosira pseudonana*, 20 µg/L for *Chlorella pyrenoidosa*, 150 µg/L for *Ulothrix fimbriata* up to 980 µg/L for *Skeletonema costatum.* Few microalgal species have been reported to resist concentrations higher than 1 mg/L (Riedel, 1984; Wong and Trevors, 1988). Moreover, when adding 0.4 mg/L chromium to a natural community, the composition shifted in dominance from diatoms to green microalgae and cyanobacteria, indicating a higher susceptibility of diatoms to Cr (VI) (Patrick, 1978). Previous studies reported that exposure of *N. pelliculosa* or *P. tricornutum* to heavy metals such as chromate resulted in a significant decrease or a complete inhibition of growth (Gabbasova et al., 2017; Irving et al., 2009). Gabbasova et al. (2017) studied the effect of Cr (VI) on diatoms and reported that the biomass yield of *P. tricornutum* at 2.5, 5, 10, 15 and 25 mg/L Cr (VI) was 60, 45, 25, 20, and 10% of the control, respectively. This is in agreement with our study in which 1 mg/L Cr (VI) had no effect on the biomass yield of *P. tricornutum*, while 5 and 10 mg/L Cr (VI) resulted in a decrease of 60-80% (Table 1). This confirms that the negative effects of Cr (VI) on growth and photosynthesis of *P. tricornutum* starts in the concentration range of 1-2.5 mg/L dichromate. The mechanism underlying the different sensitivity to Cr in microalgae remains to be clarified, yet some suggestions have been made to explain it such as interference with the cell cycle, inhibition of respiration and/or photosynthesis, or loss of motility (Cervantes et al., 2001). Gabbasova et al. (2017) found that chromate may inhibit photosystem II of *P. tricornutum*. Riedell (1984) proposed that chromium toxicity in diatoms is caused by competition with sulfate. Because the concentration of sulfate in our media was high (23 mM) it might explain the relatively low toxicity of chromium in the marine diatoms we investigated.

3.2 Optimizing growth

Both strains were tested for growth on three different media, the standard MDV, T+, and ASNIII media. The highest OD_{600} value was obtained when growing on the standard MDV medium (Table 1). T+ medium yielded only slightly

lower biomass compared to MDV but ASNIII decreased the growth yield by nearly 50% and 65% in *P. tricornutum* and *N. pelliculosa*, respectively. The diatoms for subsequent experiments were therefore cultured in MDV medium. The nitrogen source of all three media was $NaNO_3$ at a concentration of 6 mM and all three containing 150 µM silicate to support frustule production by diatoms. However, there are several differences between the three media with regards to their ingredients (Mg, Ca, K, P, S, carbonate, trace element and supplementary vitamin mix) and their respective concentrations (supplementary material) that may affect the growth yield. The two prominent differences between MDV and T+ versus ASNIII are that the first two contain a supplementary vitamin mix and higher carbonate concentrations (~10 times). Previous studies demonstrated that both factors can stimulate higher biomass production by microalgae (Danesh et al., 2018; Eppley, 1977; Lohman et al., 2015).

Increasing the growth temperature to 23 °C and 27 °C for respectively *P. tricornutum* and *N. pelliculosa* resulted in an increase in biomass (Table 1). Above these temperatures the diatoms did not grow. The biomass increased with approximately 0.1 OD_{600} units at their maximum growth temperature relative to the routinely applied growth temperature of 14 °C.

For *P. tricornutum*, increasing the light intensity from 30 to 50 µmol $m^{-2}s^{-1}$ and from 50 to 80 µmol $m^{-2}s^{-1}$ showed no significant change in OD_{600}, however, comparing light intensity at 30 to 80 µmol $m^{-2}s^{-1}$ showed a slight decrease in biomass. At 30 µmol $m^{-2}s^{-1}$ the biomass was approximately 0.05 to 0.06 OD_{600} units higher. For *N. pelliculosa* there was no significant difference in biomass yield at any of the tested light intensities.

Previous studies demonstrated that the type of nitrogen source could significantly affect growth yield and lipid production in microalgae. For example, while sodium nitrate or sodium nitrite can stimulate growth in many microalgal species, non-toxic ammonium (NH_4^+) can dissociate to the more toxic form of ammonia (NH_3) (Qiao et al., 2016; Sharma et al., 2012; Zhu et al., 2016). The standard medium contains nitrate (6 mM). The same concentration of nitrite did not affect growth of *P. tricornutum* and *N. pelliculosa*. Urea did not affect growth of *P. tricornutum* but decreased the growth yield of *N. pelliculosa*. Ammonium nitrate decreased the growth yield by 0.15 OD_{600} units for *N. pelliculosa*, while *P. tricornutum* did not grow very well on this nitrogen source (Table 1). Use of either 6 mM nitrate or nitrite is possible but ammonium at this concentration appears to be toxic (Admiraal, 1977) due to pH effects (Fidalgo Paredes et al., 1995). Although urea has not been reported to be as toxic as ammonia, previous studies demonstrated a toxic effect at high concentrations (Admiraal, 1977; Yongmanitchai and Ward, 1991).

Growth yield of both strains is furthermore positively correlated with $NaNO_3$ concentration. Without $NaNO_3$ growth halted. *P. tricornutum* and *N. pelliculosa* required respectively at least 3 mM and 4.5 mM nitrate for highest growth yield. Higher concentrations of $NaNO_3$ did not further increase the growth yield (Table 1).

Another important nutrient for diatoms is silicate, essential for the formation of their frustules (Coombs et al., 1967). The effect of silicate concentration was combined with different concentrations of $NaNO_3$ (Table 1). Silicate deprivation had no significant growth effect on *P. tricornutum* and decreased growth yield only at low $NaNO_3$ (0.6 mM). In contrast, the growth yield of *N. pelliculosa* was dramatically decreased (to ~0.1 OD_{600} units) independent on the concentration of $NaNO_3$. At 150 µM silicate, growth yield of *N. pelliculosa* was ~0.27 OD_{600} units at 0.6 mM $NaNO_3$ and was higher at 3 and 6 mM $NaNO_3$. *N. pelliculosa* obviously requires silicate for growth. This species is known to be sensitive to silicate deprivation. Without silicate, the growth of *N. pelliculosa* ceases and cell metabolism is restricted to maintenance until silicate becomes available (Coombs et al., 1967). In contrast, *P. tricornutum* is the only reported diatom species so far, that grows well without silicate (Lewin, 1958). *P. tricornutum* has a unique morphological plasticity and only forms a silicified frustule in its oval form. This morphology is mostly found in intertidal sediments and is essential for gliding motility and general stress resistance (Borowitzka and Volcani, 1978; De Martino et al., 2007). In liquid medium frustules are not formed by *P. tricornutum*.

3.3 Effect of growth condition on Cr (IV) bioremediation

3.3.1 The role of extracellular polymeric substances (EPS) in dichromate removal

The mechanism of Cr removal in *P. tricornutum* and *N. pelliculosa* is not precisely known. In general, removal of heavy metals such as dissolved Cr (VI) from the medium could be through sequestration, either by e.g. active uptake by the cells or by biosorption to (extracellular) substances produced by the cells, or by enzymatic reduction of the compound (Mantzorou et al., 2018; Perales-Vela et al., 2006). However, previous studies suggest that biosorption to extracellular materials produced by the microorganisms (such as phytochelatins) is an important mechanism for bioremediation of heavy metals by microalgae including diatoms such as *P. tricornutum* (Bertrand and Poirier, 2005; Cassin et al., 2018; Morelli and Scarano, 2001; Mota et al., 2016; Perales-Vela et al., 2006; Richards and Mullins, 2013).

In order to determine the underlying mechanism (enzymatic reduction, active uptake, or biosorption), chromium removal by living diatoms was compared with heat-killed (autoclaved) cell suspensions (Okeke, 2008). The living cultures of *P. tricornutum* and *N. pelliculosa* removed up to 30-35% Cr (VI) (Fig. 2A & B). However, when the autoclaved cell suspensions were exposed for three days (to ensure equilibrium) to 1 mg/L of Cr (VI), the amount of Cr (VI) removed was 76% and 72% of the removal by living cultures of *P. tricornutum* and *N. pelliculosa*, respectively (Table 2). The positive correlation of Cr (VI) removal with biomass, the removal of Cr (VI) by heat-killed cells, and the difference in removal after short or long time exposure all suggest a minimal contribution of metabolism-dependent Cr (VI) (active uptake and enzymatic reduction) to the bioremediation process, and that Cr (VI) removal is rather caused by biosorption (Okeke, 2008; Okeke et al., 2008).

Biosorption to the EPS would be a possibility to immobilize toxic metal ions (Cassin et al., 2018; Mantzorou et al., 2018; Pereira et al., 2011; Sharma et al., 2008). Since EPS is continuously produced and a part of it is released into the environment, toxic metal ions may be immobilized for the long term (Mota et al., 2016). To test whether EPS from *P. tricornutum* and *N. pelliculosa* is capable of binding Cr (VI), EPS was extracted from the diatom cultures and tested for chromium removal. The experiment was performed in accordance to Sharma et al. (2008) and chromium removal was measured after 3 days. Although extraction may change the conformation of the EPS, previous studies demonstrated that the extracted EPS still retain its heavy metal biosorption capacity (Mota et al., 2016; Sharma et al., 2008). Our results show that for *P. tricornutum* EPS, the amount of Cr (VI) removed was 32% and 24% of the removal by dead and live cells, respectively, and for *N. pelliculosa* EPS, these numbers were respectively 37% and 27 % (Table 2). This indicates that EPS plays a major role in the bioremoval process.

Bioremediation through binding to EPS may be attributed to phytochelatins (Cassin et al., 2018; Mantzorou et al., 2018) but may also be the result of the presence of negatively charged groups that bind the positively charged metallic ions (Mota et al., 2016). Carboxyl, hydroxyl, phosphoryl, sulfhydryl, and amino functional groups have been reported to be involved in the metal binding process, depending on the copiousness, accessibility and chemical state of the sites, and on the affinity between the metal and biosorption sites of the EPS (De Philippis et al., 2011). Moreover, Mota et al. (2016) argued that hexavalent chromium ion has a 6 valence positive charge and therefore has a strong affinity for binding to EPS. Although other positively charged metal ions are present in MDV medium, it has been argued that some heavy metal cations such as Cr (VI) are capable of replacing others that are bound to EPS through a cation exchange (Irving et al., 2009; Mota et al., 2016).

Chromium removal normalized to OD_{600} under the different cultivation conditions varied between 30% and 40 % per OD600 unit (Fig. 2A & B, panels a-h). In the short term vs long term exposure experiment, the effect of 15 versus 1 day exposure to 1 mg Cr (V)/L was compared. For both strains, short exposure led to a 20 to 25% Cr (VI) bioremediation compared to the 40% that was observed after 15 days incubation and this was independent of the amount of $NaNO_3$ (3 versus 6 mM) used in this experiment.

For improving Cr (VI) removal we investigated several growth conditions. Compared to the standard growth conditions only increasing temperature from 14^o to 23^o (*P. tricornutum*) and 27^oC (*N. pelliculosa*) improved Cr (VI) removal, while other growth parameters, including lower light intensity, using ammonium nitrate as nitrogen source, lower nitrate concentration, or silicate limitation (*N. pelliculosa*) in fact affected Cr (VI) removal negatively (Fig. 1). Sodium azide at the tested concentrations did not change biomass yield nor chromium bioremediation. This indicates that the amount of biomass appeared to be important for chromium removal. Growth parameters that led to a higher biomass also favored chromium bioremediation but conditions that decreased biomass yield affected chromium removal in a negative way.

3.4 Assessing and optimization of lipid production in the presence of chromium (VI)

Cultures were analyzed for their lipid content when grown in the presence of 1 mg/L Cr (VI). Biomass-normalized lipid content under standard growth conditions in the absence of Cr (VI) was ~40 µg triolein/ OD_{600} unit for *P. tricornutum* and ~ 30 µg triolein/ OD_{600} unit for *N. pelliculosa*. Addition of Cr (VI) at 1 mg/L to *P. tricornutum* cultures and in 1 – 5 mg/L to *N. pelliculosa* cultures did not significantly affect OD_{600} normalized lipid production. Lipid production in *N. pelliculosa* and *P. tricornutum* responded in the same way to the growth conditions. Differences were only associated with conditions when the growth of one of the two diatoms was impaired. Lipid content in cultures of *P. tricornutum* was negligibly higher when grown in T+ medium but significantly lower in ASN (III) relative to MDV (Fig. 2A & B panel b). Previous studies on lipid production by microalgae demonstrated that presence of vitamin mix in the medium (in our study MDV and T+ medium) not only supported higher biomass production but also it led to higher lipid accumulation (Danesh et al., 2018; Lohman et al., 2015).

Increasing the growth temperature of the *P. tricornutum* cultures from 14 to 23 °C resulted in a 43% increase in lipid accumulation. For *N. pelliculosa*, which grew at its highest rate at 27 °C a nearly 300 % increase in lipid content was observed relative to 14 °C grown cells, from 29 to 114 µg triolein/ OD_{600} unit.

Biomass and lipid accumulation exponentially increase with temperature (Sharma et al., 2012; Zhu et al., 2016). During growth, cells accumulate little lipid but this changes during the stationary phase when lipid accumulates to a high amount (Sharma et al., 2012; Zhu et al., 2016). Previous studies argued that a faster growth rate means a higher biomass production and, hence, a higher rate of nutrient (nitrogen) consumption. This leads to nitrogen depletion triggering a higher lipid accumulation (KaiXian and Borowitzka, 1993; Zhu et al., 2016). Our results suggest that nutrition limitation (most likely nitrate limitation) probably happens faster at 23 and 27°C and when the initial nitrogen (nitrate) concentration was 3 mM. Under these conditions, *P. tricornutum* and *N. pelliculosa* produced sufficient biomass containing a higher amount of lipid compared to standard conditions (Fig. 2).

Different microalgae species achieve their highest lipid content at different light intensities, which is due to their difference in light use efficiencies (Zhu et al., 2016). Limiting light or too high intensities may hinder the growth of microalgae. Low light intensities result in low biomass yield and lipid accumulation (Sharma et al., 2012; Vitova et al., 2015; Zhu et al., 2016), while high light intensity may cause photoinhibition, damages the photosystems and therefore results in a lower lipid accumulation (KaiXian and Borowitzka, 1993; Sharma et al., 2012; Vitova et al., 2015; Zhu et al., 2016).

In our study, we showed that maintaining light intensity at 55 µmol $m^{-2}s^{-1}$ or higher is essential to maintain lipid accumulation. In comparison, at 30 µmol $m^{-2}s^{-1}$ lipid accumulation is decreased by more than 50% in *P. tricornutum* and with 25% in *N. pelliculosa*, while increasing the light intensity to 80 µmol $m^{-2}s^{-1}$ had no effect on lipid accumulation. Increased lipid content in *P. tricornutum* has been reported in response to increasing light intensities (KaiXian and Borowitzka, 1993; Zhu et al., 2016).

The source of nitrogen (sodium nitrate, sodium nitrite, urea, and ammonium nitrate) was not important for the biomass normalized lipid production (Fig. 2 A & B, panel e), while decreasing the $NaNO_3$ concentration increased the biomass-normalized lipid content dramatically (Fig. 2 A & B, panel f). A nearly 1100% increase in biomass-normalized lipid content was found in *P. tricornutum* when grown at 0.6 mM $NaNO_3$, resulting in a lipid content of 663 µg triolein/1 OD600 units. For *N. pelliculosa*, a more than 400% increase to 517 µg triolein/1 OD600 units was found at 0.6 mM $NaNO_3$ relatively to cultures that were grown at 6 mM nitrate. However, despite the higher lipid content, the total biomass of cultures growing at 0.6 mM decreased by more than 50%. Nutrient starvation is known as the most successful and widely used strategy to enhance lipid productivity. Increasing sodium nitrate concentration significantly enhanced biomass production by *P. tricornutum* and resulted in decreased lipid

content/biomass (Yodsuwan et al., 2017) and using low nitrogen concentrations results in a dramatic decrease of biomass, but with high lipid content (KaiXian and Borowitzka, 1993).

In this study, the desirable condition is a trade-off between a high lipid content and a good chromium bioremoval. Three mM nitrate and 23 and 27 °C for *P. tricornutum* and *N. pelliculosa*, respectively, shortened the time to the stationary phase and nutrition (nitrogen) limitation triggering lipid accumulation, while sufficient chromium biosorption occurred.

4. Conclusion

Compared to other microorganisms, microalgae and cyanobacteria are not exceptional for their bioremediation capacities, but they have several attributes that make them an interesting and advantageous alternative for bioremediation. They are the primary producers in many ecosystems and many strains grow in marine and brackish habitats. This avoids the requirement of fresh water and arable land for biotechnological applications. These organisms can even be used for multiple purposes such as combining heavy metal bioremediation and lipid production (Perales-Vela et al., 2006; Richards and Mullins, 2013; Wilde and Benemann, 1993). These features raised an increasing interest in the use of these phototrophic microorganisms for biotechnological applications, including bioremediation.

We showed that *P. tricornutum* and *N. pelliculosa* not only produced sufficient biomass and accumulated large amounts of lipid but are also capable of bioremediation of hexavalent chromium at concentrations toxic for human (1 mg/L). The positive correlation of Cr (VI) removal with biomass, effective removal in heat-inactivated cells and the difference in removal after short or long time exposure and biosorption of chromium cations by extracted EPS hinted to a cell biosorption mechanism with EPS capture as the main mechanism used by the two strains for bioremediation process.

The chromium bioremoval capacity of these strains was correlated to the amount of biomass. Biomass production was enhanced at an increased temperature. However, a high lipid content that was triggered by nutrient (nitrogen) depletion was not in favor of a high biomass production and consequently Cr bioremediation. Cr removal and lipid accumulation by these strains require optimization of the growth conditions in order to achieve both goals.

References

Admiraal, W., 1977. Tolerance of estuarine benthic diatoms to high concentrations of ammonia, nitrite ion, nitrate ion and orthophosphate. Mar. Biol. 43, 307-315.

Agency for Toxic Substances and Disease Registry (ATSDR), 2012. Toxicological profile for chromium. US Department of Health and Human Services Reports, Atlanta, USA.

American Public Health Association (APHA), 1989 and 1996. APHA methods: standard methods for the examination of water and wastewater. Standard method 3500-Cr, Washington DC.

Barrera-Díaz, C.E., Lugo-Lugo, V., Bilyeu, B., 2012. A review of chemical, electrochemical and biological methods for aqueous Cr(VI) reduction. J. Hazard. Mat. 223, 1-12.

Bertrand, M., Poirier, I., 2005. Photosynthetic organisms and excess of metals. Photosynthetica 43, 345-353.

Borowitzka, M.A., Volcani, B.E., 1978. The polymorphic diatom *Phaeodactylum tricornutum*: ultrastructure of its morphotypes. J. Phycol. 14, 10-21.

Bozarth, A., Maier, U.-G., Zauner, S., 2009. Diatoms in biotechnology: modern tools and applications. Appl. Microbiol. Biotechnol. 82, 195-201.

Canadian Federal Environmental Quality Guidelines (FEQGs), 2017. Canadian Environmental Protection Act, 1999, Hexavalent Chromium. FEQGs, Canada

Cassin, R., Nonomura, A.M., Mayzaud, P., Noüe, J.d.l., Duerr, E.O., Redalje, D.G., 2018. Algae as ideal waste removers: biochemical pathways, in: Huntley, M. E. (Eds), Biotreatment of Agricultural Wastewater. CRC Press, Boca Raton, pp. 91-110.

Cervantes, C., Campos-García, J., Devars, S., Gutiérrez-Corona, F., Loza-Tavera, H., Torres-Guzmán, J.C., Moreno-Sánchez, R., 2001. Interactions of chromium with microorganisms and plants. FEMS Microbiol. Rev. 25, 335-347.

Coombs, J., Darley, W., Holm-Hansen, O., Volcani, B., 1967. Studies on the biochemistry and fine structure of silica shell formation in diatoms. Chemical composition of *Navicula pelliculosa* during silicon-starvation synchrony. Plant Physiol. 42, 1601-1606.

Danesh, A.F., Mooij, P., Ebrahimi, S., Kleerebezem, R., Loosdrecht, M.v., 2018. Effective role of medium supplementation in microalgal lipid accumulation. Biotechnol. Bioeng. 115, 1152-1160.

De Martino, A., Meichenin, A., Shi, J., Pan, K., Bowler, C., 2007. Genetic and phenotypic characterization of *Phaeodactylum tricornutum* (Bacillariophyceae) accessions. J. Phycol. 43, 992-1009.

De Philippis, R., Colica, G., Micheletti, E., 2011. Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process. Appl. Microbiol. Biotechnol. 92, 697-708.

Dönmez, G., Aksu, Z., 2002. Removal of chromium(VI) from saline wastewaters by *Dunaliella* species. Process Biochem. 38, 751-762.

Eppley, R., 1977. The growth and culture of diatoms, in: Werner, D. (Eds.), The Biology of Diatoms. University of California Press, Berkeley and New York, p. 24-64.

Fidalgo Paredes, P., Cid, Á., Abalde, J., Herrero, C., 1995. Culture of the marine diatom *Phaeodactylum tricornutum* with different nitrogen sources: Growth, nutrient conversion and biochemical composition. Cahiers. Biol. Mar. 36, 165-173.

Gabbasova, D.T., Matorin, D.N., Konyukhov, I.V., Seifullina, N.K., Zayadan, B.K., 2017. Effect of chromate ions on marine microalgae *Phaeodactylum tricornutum*. Microbiology+ 86, 64-72.

Greenwood, N., Earnshaw, A., 1997. Chemistry of the Elements, second ed. Butterworth-Heinemann. London.

Irving, E.C., Baird, D.J., Culp, J.M., 2009. Cadmium toxicity and uptake by mats of the freshwater diatom: *Navicula pelliculosa* (bréb) hilse. Arch. Environ. Con. Toxicol. 57, 524-530.

KaiXian, Q., Borowitzka, M.A., 1993. Light and nitrogen deficiency effects on the growth and composition of *Phaeodactylum tricornutum*. Appl. Biochem. Biotech. 38, 93-103.

Kaur, S., 2014. Genetic and biotechnological development of the pennate marine diatom *Phaeodactylum tricornutum* for high-value bioproducts and carbon bio-mitigation. Ph.D. thesis. College of Science, National University of Ireland. Galway.

Laurens, L.M.L., Markham, J., Templeton, D.W., Christensen, E.D., Wychen, S.V., Vadelius, E.W., Chen-Glasser, M., Dong, T., Davis, R., Pienkos, P.T., 2017. Development of algae biorefinery concepts for biofuels and bioproducts; a perspective on process-compatible products and their impact on cost-reduction. Ener. Environ. Sci. 10, 1716-1738.

Lewin, J.C., 1955. Silicon metabolism in diatoms: iii. Respiration and silicon uptake in *Navicula pelliculosa*. J. Gen. Physiol. 39, 1-10.

Lewin, J.C., 1958. The taxonomic position of *Phaeodactylum tricornutum*. Microbiology+ 18, 427-432.

Lohman, E.J., et al., Optimized inorganic carbon regime for enhanced growth and lipid accumulation in *Chlorella vulgaris*. Biotechnol. Biofuels 2015. 82, p. 1-13.

Mantzorou, A., Navakoudis, E., Paschalidis, K., Ververidis, F., 2018. Microalgae: a potential tool for remediating aquatic environments from toxic metals. Int. J. Environ. Sci. Technol. 15, 1-16.

Mishra, S.K., Suh, W.I., Farooq, W., Moon, M., Shrivastav, A., Park, M.S., Yang, J.-W., 2014. Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method. Bioresource Technol. 155, 330-333.

Morelli, E., Scarano, G., 2001. Synthesis and stability of phytochelatins induced by cadmium and lead in the marine diatom *Phaeodactylum tricornutum*. Mar. Environ. Res. 52, 383-395.

Mota, R., Rossi, F., Andrenelli, L., Pereira, S.B., Philippis, R.D., Tamagnini, P., 2016. Released polysaccharides (RPS) from *Cyanothece* sp. CCY 0110 as biosorbent for heavy metals bioremediation: interactions between metals and RPS binding sites. Appl. Microbiol. Biotechnol. 100, 7765-7775.

Okeke, B.C., 2008. Bioremoval of hexavalent chromium from water by a salt tolerant bacterium, *Exiguobacterium* sp. GS1. J. Ind. Microbiol. Biotechnol. 35, 1571-1579.

Okeke, B.C., Laymon, J., Crenshaw, S., Oji, C., 2008. Environmental and kinetic parameters for Cr(VI) bioreduction by a bacterial monoculture purified from Cr(vi)-resistant consortium. Biol. Trace Elem. Res. 123, 229-241.

Patrick, R., 1978. Effects of trace metals in the aquatic ecosystem: the diatom community, base of the aquatic food chain, undergoes significant changes in the presence of trace metals and other alterations in water chemistry. Am. Sci. 66, 185-191.

Perales-Vela, H.V., Peña-Castro, J.M., Cañizares-Villanueva, R.O., 2006. Heavy metal detoxification in eukaryotic microalgae. Chemosphere 64, 1-10.

Pereira, S., Micheletti, E., Zille, A., Santos, A., Moradas-Ferreira, P., Tamagnini, P., De Philippis, R., 2011. Using extracellular polymeric substances (EPS)-producing cyanobacteria for the bioremediation of heavy metals: do cations compete for the EPS functional groups and also accumulate inside the cell? Microbiology+ 157, 451-458.

Petrilli, F.L., De Flora, S., 1977. Toxicity and mutagenicity of hexavalent chromium on *Salmonella typhimurium*. Appl. Environ. Microbiol. 33, 805-809.

Qiao, H., Cong, C., Sun, C., Li, B., Wang, J., Zhang, L., 2016. Effect of culture conditions on growth, fatty acid composition and DHA/EPA ratio of *Phaeodactylum tricornutum*. Aquaculture 452, 311-317.

Richards, R.G., Mullins, B.J., 2013. Using microalgae for combined lipid production and heavy metal removal from leachate. Ecol. Modell. 249, 59-67.

Riedel, G.F., 1984. Influence of salinity and sulfate on the toxicity of chromium (VI) to the estuarine diatom *Thalassiosira pseudonana*. J. Phycol. 20, 496-500.

Sarthou, G., Timmermans, K.R., Blain, S., Tréguer, P., 2005. Growth physiology and fate of diatoms in the ocean: a review. J. Sea Res. 53, 25-42.

Sen, M., Dastidar, M.G., 2010. Chromium removal using various biosorbents. Iran J. Environ. Health. 7, 182-190.

Shanker, A.K., Cervantes, C., Loza-Tavera, H., Avudainayagam, S., 2005. Chromium toxicity in plants. Environ. Int. 31, 739-753.

Sharma, K.K., Schuhmann, H., Schenk, P.M., 2012. High lipid induction in microalgae for biodiesel production. Energies 5, 1532-1553.

Sharma, M., Kaushik, A., Somvir, Bala, K., Kamra, A., 2008. Sequestration of chromium by exopolysaccharides of *Nostoc* and *Gloeocapsa* from dilute aqueous solutions. J. Hazard. Mater. 157, 315-318.

Smetacek, V., 1999. Diatoms and the ocean carbon cycle. Protistology 150, 25-32.

Staats, N., De Winder, B., Stal, L., Mur, L., 1999. Isolation and characterization of extracellular polysaccharides from the epipelic diatoms *Cylindrotheca closterium* and *Navicula salinarum*. Eur. J. Phycol. 34, 161-169.

Straif, K., Benbrahim-Tallaa, L., Baan, R., Grosse, Y., Secretan, B., El Ghissassi, F., Bouvard, V., Guha, N., Freeman, C., Galichet, L., Cogliano, V., 2009. A review of human carcinogens--Part C: metals, arsenic, dusts, and fibres. The Lancet. Oncology 10, 453-454.

R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/.

U.S. Department of Health and Human Services (HHS), 2016. Chromium, Office of Dietary Supplements, US National Institutes of Health.

U.S. Department of Health and Human Services (HHS), 2008. Toxicology and carcinogenesis studies of sodium dichromate dihydrate (Cas No. 7789-12-0) in F344/N rats and B6C3F1 mice (drinking water studies), National Toxicology Program technical report series reports. US National Institutes of Health.

Velma, V., Vutukuru, S.S., Tchounwou, P.B., 2009. Ecotoxicology of hexavalent chromium in freshwater fish: a critical review. Rev. Environ. Health 24, 129-145.

Vitova, M., Bisova, K., Kawano, S., Zachleder, V., 2015. Accumulation of energy reserves in algae: from cell cycles to biotechnological applications. Biotechnol. Adv. 33, 1204-1218.

Wilde, E.W., Benemann, J.R., 1993. Bioremoval of heavy metals by the use of microalgae. Biotechnol. Adv. 11, 781-812.

Wong, P. and Trevors, J., 1988. Chromium toxicity to algae and bacteria, in: Nriagu, J.O., Nieboer, E. (Eds), Chromium in the natural and human environments. Wiley. New York. p. 305-315.

Yao, J., Tian, L., Wang, Y., Djah, A., Wang, F., Chen, H., Su, C., Zhuang, R., Zhou, Y., Choi, M.M.F., Bramanti, E., 2008. Microcalorimetric study the toxic effect of hexavalent chromium on microbial activity of Wuhan brown sandy soil: an in vitro approach. Ecotox. Environ. Safety 69, 289-295.

Yodsuwan, N., Sawayama, S., Sirisansaneeyakul, S., 2017. Effect of nitrogen concentration on growth, lipid production and fatty acid profiles of the marine diatom *Phaeodactylum tricornutum*. Agric. Nat. Res. 51, 190-197.

Yongmanitchai, W., Ward, O.P., 1991. Growth of and omega-3 fatty acid production by *Phaeodactylum tricornutum* under different culture conditions. Appl. Environ. Microbiol. 57, 419-425.

Zhitkovich, A., 2011. Chromium in drinking water: sources, metabolism, and cancer risks. Chem. Res. Toxicol. 24, 1617-1629.

Zhu, L.D., Li, Z.H., Hiltunen, E., 2016. Strategies for lipid production improvement in microalgae as a biodiesel feedstock. BioMed Res. Int. 2016, 1-8.

Figure1

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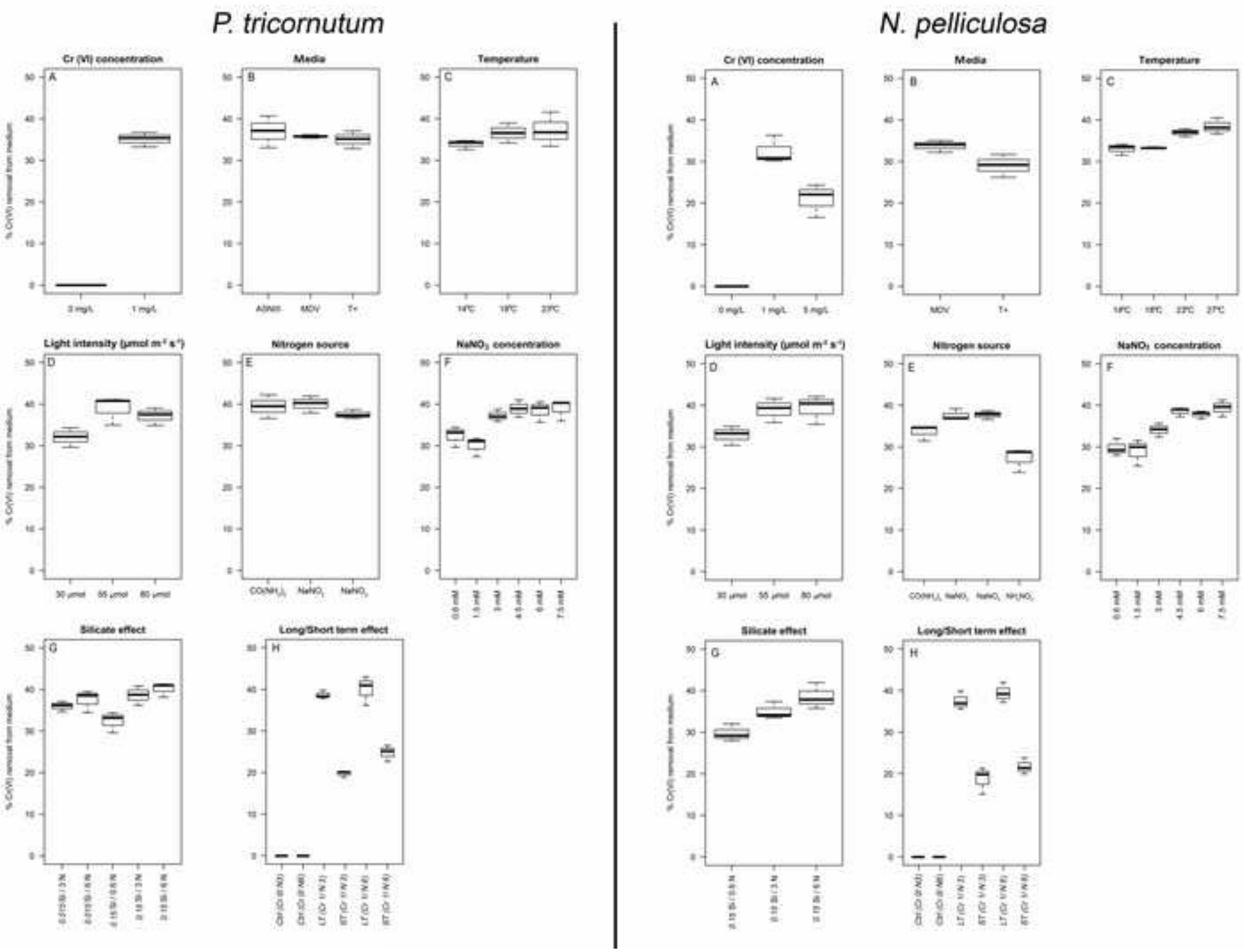

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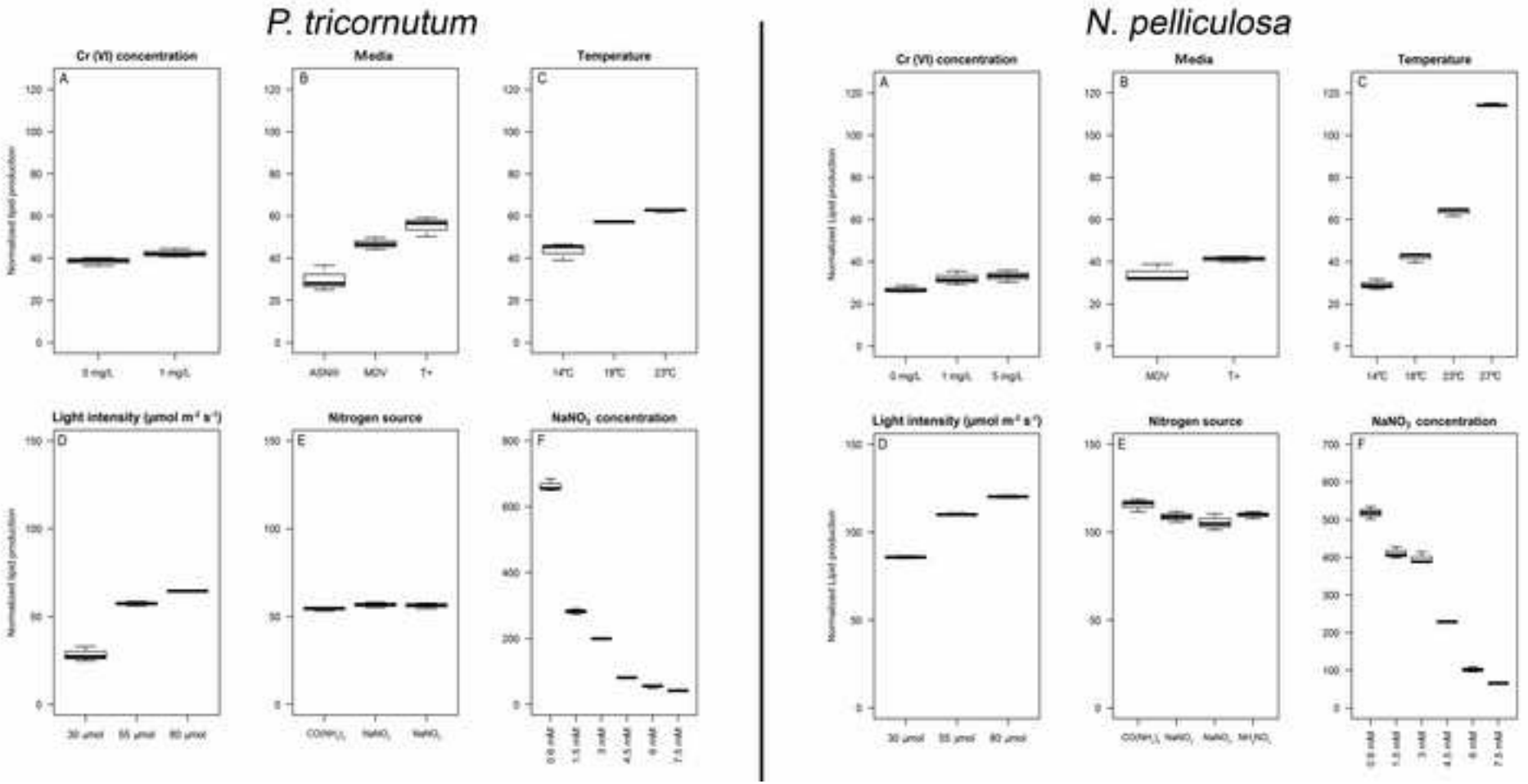

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Figure 1. Effect of growth condition on Cr(VI) removal

Figure 2. Effect of growth condition on lipid production

Table

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Table 1. Biomass production yield by *P. tricornutum* and *N. pelliculosa* at different growth variations

Experiment	Variations	*P. tricornutum*	*N. pelliculosa*
		OD_{600} ± SD[a]	OD_{600}
Cr (VI) tolerance	0	0.491 ± 0.006	0.474 ± 0.014
	50 µg/L	0.496 ± 0.007	0.483 ± 0.030
	100 µg/L	0.506 ± 0.011	0.490 ± 0.016
	500 µg/L	0.505 ± 0.015	0.507 ± 0.007
	1 mg/L	0.479 ± 0.006	0.477 ± 0.011
	5 mg/L	0.196 ± 0.004	0.318 ± 0.003
	10 mg/L	0.096 ± 0.002	0.191 ± 0.003
Media	MDV	0.477 ± 0.010	0.462 ± 0.019
	T+	0.419 ± 0.016	0.417 ± 0.010
	ASNIII	0.246 ± 0.020	0.177 ± 0.020
Temperature (°C)	14	0.477 ± 0.010	0.461 ± 0.022
	18	0.491 ± 0.012	0.492 ± 0.010
	23	0.537 ± 0.012	0.521 ± 0.006
	27	NG [b]	0.554 ± 0.011
	30	NG	NG
	33	NG	NG
	37	NG	NG
Light intensity (µmol $m^{-2}s^{-1}$)	30	0.573 ± 0.004	0.580 ± 0.010
	55	0.550 ± 0.015	0.562 ± 0.013
	80	0.519 ± 0.009	0.524 ± 0.009
Nitrogen source	Sodium nitrate	0.566 ± 0.009	0.572 ± 0.009
	Sodium nitrite	0.556 ± 0.008	0.558 ± 0.006
	Urea	0.552 ± 0.010	0.509 ± 0.007
	Ammonium nitrite	0.073 ± 0.001	0.423 ± 0.009
Nitrogen concentration (mM)	0.0	NG	NG
	0.6	0.275 ± 0.009	0.268 ± 0.010
	1.5	0.467 ± 0.004	0.426 ± 0.011
	3.0	0.522 ± 0.009	0.510 ± 0.008
	4.5	0.541 ± 0.012	0.558 ± 0.013
	6.0	0.536 ± 0.017	0.568 ± 0.014
	7.5	0.556 ± 0.010	0.561 ± 0.005
Silicate	150 µM Silicate (6.0 mM nitrate)	0.534 ± 0.007	0.555 ± 0.019
	15 µM Silicate(6.0 mM nitrate)	0.517 ± 0.007	0.111 ± 0.013
	150 µM Silicate (3.0 mM nitrate)	0.509 ± 0.004	0.506 ± 0.006
	15 µM Silicate(3.0 mM nitrate)	0.514 ± 0.005	0.085 ± 0.004
	150 µM Silicate (0.6 mM nitrate)	0.275 ± 0.009	0.268 ± 0.010
	15 µM Silicate(0.6 mM nitrate)	0.104 ± 0.006	0.070 ± 0.002
Sodium azide	0 µM Sodium azide (6.0 mM nitrate)	0.536 ± 0.013	0.543 ± 0.007
	10 µM Sodium azide (6.0 mM nitrate)	0.529 ± 0.010	0.570 ± 0.010
	25 µM Sodium azide (6.0 mM nitrate)	0.532 ± 0.007	0.524 ± 0.011
	50 µM Sodium azide (6.0 mM nitrate)	0.544 ± 0.018	0.533 ± 0.010
	100 µM Sodium azide (6.0 mM nitrate)	0.538 ± 0.022	0.553 ± 0.017
	0 µM Sodium azide (3.0 mM nitrate)	0.508 ± 0.004	0.511 ± 0.011
	10 µM Sodium azide (3.0 mM nitrate)	0.500 ± 0.003	0.510 ± 0.005

25 µM sodium azide (3.0 mM nitrate)	0.497 ± 0.007	0.514 ± 0.008
50 µM sodium azide (3.0 mM nitrate)	0.518 ± 0.006	0.496 ± 0.003
100 µM sodium azide (3.0 mM nitrate)	0.503 ± 0.005	0.489 ± 0.004

[a] SD = Standard deviation

[b] NG = No growth observed

Table 2. Cr(VI) biosorption by living cultures, heat-killed cell suspension, and EPS extract of *P. tricornutum* and *N. pelliculosa*

Experiment	Cr(VI) bioremediation[*] ± SD[a] (%)	
	P. tricornutum	***N. pelliculosa***
living cultures (A)	35 ± 1	32 ± 2
heat-killed cells (B)	27 ± 2	23 ± 1
EPS extract (C)	9 ± 0	9 ± 0
(B/A) * 100	76	72
(C/A) * 100	24	27
(C/B) * 100	32	37

[a] SD = Standard deviation

[*] Biomass-normalized Cr(VI) bioremediation

Supplementary Materials, List of the media, their ingredients, and preparation procedure.

The main procedure is addressed in the following pages. The only difference is that T+ and ASNIII media are supplemented with 0.15 mM silicate in order to support diatom growth. The second changes is that The Nitrate concentration of T+ and ASNII was adjusted at 6 mM.

MDV

Minerals	Stock Solutions (g/L)	Quantity (mL Stock/L Media)	Molarity (mM)
NaCl	241	100	400
$MgCl_2 \cdot 6H_2O$	435	20	43
KCl	54	10	7.2
Na_2SO_4	32	100	23
$CaCl_2 \cdot 2H_2O$	160	10	11

Adjust to 900mL with mQ water and autoclave.

After cooling, add the following filter sterilized (0.2 µm) components to complete the medium:

Minerals	Stock Solutions (g/L)	Quantity (mL Stock/L Media)	Molarity (mM)
$NaNO_3$	100	5	6
$NaHCO_3$	18	10	2
$NaH_2PO_4 \cdot H_2O$	6.9	1	0.05
$Na_2SiO_3 \cdot 9H_2O$	21.3	2	0.15
Citrate mix	See recipe below	10	-
Trace metal mix	See recipe below	1	-
Vitamins 8 Mix	See recipe below	1	-
M2	See recipe below	1	-

For solid medium use 7g/L of agarose. Sterilize the agarose separately in 550 ml of milliQ water. In this case the mineral solution is filled up to 400 ml.

Trace Metal Mix:

Trace metals	Stock1 (g/L)	Trace metal mix (Stock1 mL/L)
$CuSO_4 \cdot 5\ H_2O$	9.8	1
$ZnSO_4 \cdot 7H_2O$	22	1
$CoCl_2 \cdot 6H_2O$	10	1
$MnCl_2 \cdot 4H_2O$	18	1
$Na_2MoO_4 \cdot 2H_2O$	6.3	1
$Na_2SeO_3 \cdot 5H_2O$	0.016	0.1

Prepare apart a stock solution for each Trace metal (Stock1) and use the quantity indicated for the final Trace Metal Mix

Citrate Mix:

Trace metals	Quantity g/L
$C_6H_8O_7 \cdot H_2O$	0.3
Fe-NH_4-citrate	0.36

M2:

Trace metals	Quantity (g/L)
KBr	39
$SrCl_2 \cdot 6H_2O$	10
$AlCl_3 \cdot 6H_2O$	0.014
LiCl	0.003
KI	0.010
H_3BO_3	11
RbCl	0.03

Vitamins 8 mix:

Vitamins	Stock 1 (g/100mL)	Vitamins 8 mix (Stock1 mL/100mL)
Biotin*	0.004	0.1
Thiamine-HCl	0.02	10
Cyanocobalamin	0.08	0.1
Folic acid*	0.008	0.1
Inositol	0.02	1
Nicotinic acid	0.04	1
Thymine*	0.012	1
Ca-d-pantothenate	0.04	1

*Dissolve first in 1N NaOH and then bring to volume with mQ water.

Prepare apart a stock solution for each Vitamin (Stock1) and use the quantity indicated for the final Vitamins 8 mix.

T^+

(Modified from: Chen Y. B., Zehr J. P., Mellon M., 1996. Growth and nitrogen fixation of the diazotrophic filamentous non-heterocystous cyanobacterium *Trichodesmium* sp. IMS 101 in defined media: evidence for a circadian rhythm . *J Phycol*. 32: 916-923.)

Minerals	Stock Solutions (g/L)	Quantity (mL Stock/L Media)	Final Concentration (mM)
NaCl	245.45	100	420
$MgCl_2 \cdot 6\ H_2O$	406.6	10	20
KCl	74.60	10	10
$MgSO_4 \cdot 7\ H_2O$	603.8	10	25
$CaCl_2 \cdot 2\ H_2O$	147	10	10

Adjust to 900mL with mQ water and autoclave.

After cooling, add the following filter sterilized (0.2 µm) components to complete the medium:

Minerals	Stock Solutions (g/L)	Quantity (mL Stock/L Media)	Final Concentration (mM)
$NaNO_3$	150	10	16
$NaHCO_3$	21	10	2.5
$K_2HPO_4 \cdot 3H_2O$	6.8	1	0.03
Na_2CO_3	26.5	0.6	0.15
Fe-NH_4-citrate	6	0.25	-
KBr	115.7	1	0.97
NaF	2.9	1	0.07
Trace Metal Mix 4	See recipe below	1	-
Trace Metal Mix	See recipe below	1	-
Vitamins3 Mix	See recipe below	1	-

Check the pH, has to be between 8.12 and 8.2.

For solid medium use 7g/L of agarose. Sterilize the agarose separately in 550 ml of milliQ water. In this case the mineral solution is filled up to 400 ml.

Trace Metal Mix 4:

Trace metals	Quantity g/L	Concentration in the final media (mM)
H_3BO_3	35.9	0.0006
$SrCl_2 \cdot 6H_2O$	17.3	0.00006
LiCl	1.1	0.00003
$Na_2SeO_3 \cdot 5H_2O$	0.5mL of a stock of 32mg/L	0.00000006

Trace Metal Mix:

Trace metals	Stock 1 (g/100mL)	Trace metal mix (Stock1 mL/L)	Concentration in the final media (mM)
EDTA	-	0.74 g	2.5
FeCl3 · 6H2O	-	0.11 g	0.0004
MnCl2 · 4H2O	0.4	1	0.00002
ZnSO4 · 7H2O	0.12	1	0.000004
CoCl2 · 6H2O	0.06	1	0.000002
Na2MoO4 · 2H2O	0.27	1	0.00001
CuSO4 · 5H2O	0.025	1	0.000001

Vitamins 3 Mix:

Vitamins	Vitamins 3 mix (quantity/100mL)
Thiamine-HCl	10 mg
d-biotin*	100 µL (from a stock of 5 mg in 10mL)
VItamin B12	100 µL (from a stock of 5 mg in 10mL)

*Dissolve first in 0.1 mL 2M NaOH. Then add 9.9 mL of mQ water.

ASN3

(Modified from: Rippka R., 1988. Isolation and purification of cyanobacteria. *Method Enzymol*. 167: 3-27.)

Minerals	Stock Solutions (g/L)	Quantity (mL Stock/L Media)	Final Concentration (mM)
NaCl	250	100	428
$MgCl_2 \cdot 6H_2O$	200	10	10
KCl	50	10	6.5
$MgSO_4 \cdot 7H_2O$	350	10	14
$CaCl_2 \cdot 2H_2O$	50	10	3
Na_3-citrate	0.6	5	0.012
Na_2-EDTA $\cdot 2H_2O$	0.1	5	0.0013
Trace metal mix (A5 + Co)	See recipe below	1	-

Adjust to 900mL with mQ water and autoclave.

After cooling, add the following filter sterilized (0.2 µm) components to complete the medium:

Minerals	Stock Solutions (g/L)	Quantity (mL Stock/L Media)	Final Concentration (mM)
$NaNO_3$	150	5	8.8
$K_2HPO_4 \cdot 3H_2O$	4	5	0.088
Na_2CO_3	20	1	0.19
Fe-NH_4-citrate	6	0.5	-
Vitamin B12 (Cyanocobalamin)	0.02	1	-

For solid medium use 7g/L of agarose. Sterilize the agarose separately in 550 ml of milliQ water. In this case the mineral solution is filled up to 400 ml.

Trace metal mix A5 + Co:

Trace metals	Quantity g/L	Concentration in the final media (mM)
H_3BO_3	2.86	0.047
$MnCl_2 \cdot 4H_2O$	1.81	0.009
$ZnSO_4 \cdot 7H_2O$	0.22	0.0007
$Na_2MoO_4 \cdot 2H_2O$	0.39	0.0016
$CuSO_4 \cdot 5H_2O$	0.08	0.0003
$Co(NO_3)_2 \cdot 6H_2O$	0.05	0.0002