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Efficiency of transgenic *Chlamydomonas reinhardtii* for removing of toxicity of cyanide compound.

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Abstract

In this study, we used the *Agrobacterium tumefaciens*–mediated transformation approach to transfer the activity of the cyanobacterial cyanase enzyme into the micro-alga, *Chlamydomonas reinhardtii*. In transgenic *C. reinhardtii* strains, the recombinant cyanase enzyme was proven to be active. In comparison to wild-type control, transgenic type demonstrated higher rates of ammonia release, reduced loss of pigmentation, and a lower percentage of growth retardation when varied amounts of cyanide (up to 200 ppm) were applied to the growth medium. Moreover, cyanase activity increases as concentration of cyanide increase especially in case of transgenic. The maximum activity was indicated in presence of 100 mg/l cyanide it reached eight folds more than wild activity at the same cyanide concentration. Results of this study provide an effective eco-friendly phytoremediation system for detoxification of cyanide using micro-alga compared to previously reported conventional system for removal of cyanide compounds, Also, some factors are taken in consideration like different pH, contact time and the transgenic type has been the priority for removal cyanide at wide range of pH with two folds more than wild type.

Keywords: Cyanide; Phytoremediation; Transgenic and *Chlamydomonas reinhar*dtii.

1. Introduction

The C ≡ N group is found in cyanide compounds. It can be found all throughout the environment. Many different kinds of species dissolved in water are found in varied physical forms. Cyanide is a very poisonous compound that is released into the environment by many industries such as coal cooking, metal plating, electronics, mining, electroplating, steel tempering, photography, acrylic plastics synthesis, drugs, dyes, plastics, and organic chemical manufacturing [1-7].

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Cyanide has a high toxicity because it can disrupt respiratory system, due to its binding to cytochrome C oxidase [8]. In this process, electron transport chain seized the cyanide binding with the iron ion in terminal electron acceptor in cytochrome C-oxidase leading to rapid declining respiration rates and inhibit ATP synthesis in mitochondria [9]. Small amounts of cyanide exposure can be deadly [10-13].

Water containing cyanide must be trearted before being discharged into the environment to safeguard the environment and water bodies. There are several conventional methods used in treating effluents containing cyanide before discharging it into the environment. The most common ones are the alkaline chlorination, sulfur oxide/air process and hydrogen peroxide process [12,14]. However, the used chemicals are hazardous since, the reagents create additional toxicity to biological ecosystem and these methods are expensive.

Various food crop, forage, and horticulture plants produce cyanogenic glucosides, which stored in vacuoles of plant and released as a nitrogen source when plant tissues are damaged. Some plant and algae such as *Cyanobacteria, Chlorella vulgaris*, *Scendesmus*, and *Nostoc muscorum* produce HCN as a defence mechanism against herbs [15]. Fungi like *Actinomycetes* and *Tricholoma* and bacteria like certain *Pseudomonas species, Chromobacterium violaceum* produce and metabolize cyanide [16].

The enzyme cyanidase (cyanide dihydratase) is primarily found in bacteria. Cyanide dihydratase and cyanide hydratase have similarity at both the amino acid and structural levels to nitrilase and nitrile hydratase enzymes [17].

Five general pathways as reported in literature for the biodegradation of cyanide, these are: hydrolytic pathway, oxidative pathway, reductive pathway, substitution/transfer pathway, and syntheses pathway [18]. First three pathways are degradation pathways in which enzymes catalyze the conversion of cyanides into simple organic or inorganic molecules and converts it to ammonia, methane, CO_2, formic acid, and carboxylic acid. Last two pathways are for the assimilation of cyanide in the microbe as nitrogen and carbon source [2].

The oxidative reactions for the biodegradation of cyanide form ammonia and carbon dioxide. Cyanide monoxygenase converts cyanide to canate, which canase then catalyzing the bicarbonate– dependent conversion of cyanate to ammonia and

carbon dioxide [19]. A second oxidative pathway utilizes cyanide dioxygenase to form carbon dioxide and ammonia directly. Cyanide detoxification by algae has been shown in only a few studies [20]. Several algae can effectively degrade cyanide as nitrogen and/or carbon source for their growth. Cyanide effluent degradation by *Scendesmus obliqus* has been examined [21].

Various parameters, such as the initial concentration of cyanide, initial cell density, and time, all had a substantial impact on cyanide detoxification by algae. Few microbes, such as the bacteria *Klebsiella oxytoca*, may use cyanide compounds as nitrogen and carbon sources to fuel their own growth. Despite cyanide compounds are very toxic, biological treatments are viable alternatives to chemical procedures without or adding new hazardous chemicals [3, 22- 24]. This works aims to elevate the rate of cyanide removal via transformed micro green alga with parallel comparison with the wild efficiency as an alternative method for bioremediation of cyanide.

2. Materials and methods

2.1 Materials

Prof. Dr. Mohammed Ismaeil, Faculty of Sciense, Mansoura University, Egypt, generously provided *Chlamydomonas reinhardtii* P.A.Dangeard strains CC–124(mt^-).

Prof. Dr. Yassin EL-Ayouty, Faculty of Science, Zagazig Univeristy, Egypt, generously provided transgenic genotype.

.*Chlamydomonas reinhardtii* culture conditions:

Transformation was carried out according to [25]. *C. reinharditii* was cultivated according to [26]. Solid Tris Acetate Phosphate (TAP) medium supplemented with 1.5 percent (w/v) agar and kanamycin (50μg/ml) was used to select transformed *C.reinhardtii* colonies. The algal cultures were incubated in growth chamber at 25 ˚C and kept under light intensity 80μmol m^{-2} s^{-1} with continuous shaking at 150 rpm in case of liquid cultures. The well-developed colonies in presence of kanamycin were selected and inoculated in sterile fresh TAP medium and allowed to grow under the previous condition of growth. The growing cultures were used for the following experiments.

2.2 Methods

2.2.1 Growth Assays of both genotypes under cyanide stress

Similar amounts of wild-type, *Chlamydomonas reinhardtii* CYN-1, *Chlamydomonas reinhardtii* CYN-2 cells have been inoculated into liquid TAP media supplemented with 0.0, 25, 50, 100, 150, and 200 mg/l potassium cyanide and cultivated for 26 days. The densities of algal cells were then measured at 665 nm **[27]** at regular interval periods (48 h). Mid log phase was estimated.

2.2.2 Cyanide degrading experiment

Distinct healthy green algal cells of *Chlamydomonas reinhartdii* were used as inoculum for the different flasks containing 50ml sterilized TAP medium. The different flasks comprise six group, three flasks for each concentration were prepared. The first group devoid cyanide (control), whereas the others having 25, 50, 100, 150 and 200 mg/l potassium cyanide. All the flasks were inoculated with 5mls of healthy algae under aseptic conditions. (1ml=0.6 optical density) and incubated for 72 hours under light intensity 80μmol m^{-2} s^{-1} at 25 $^{\circ}$C. For determination of KCN residu a volume of 5ml was taken from each group at different times (zero time, 6 h, 12 h, 24 h, 36 h, 48 h, and 72 h) for spectrophotometric estimation by isonicotinic acid-pyrazolone. It was measured at 638 nm by ultraviolet-visible spectrophotometer (YQ00302), according to [28] at regular intervals.

Amount of cyanide removal was calculated as follow:

Amount of cyanide removal (mg/l) = C_0 - C_r

Where C_0 is the initial concentration of cyanide (mg/l)

and C_r is the residual concentration of cyanide (mg/l).

2.2.3 Ammonia released by transegenic *C. reinhardtii* under cyanide stress

The approach reported by [29] with some modifications was used to release ammonia from transgenic *C. reinhardtii* at different potassium cyanide concentrations. For 12 days, transgenic *C. reinhardtii* cells and wild type were grown in liquid TAP media. Algal cells were harvested, and a weight of 0.02 g of algal cells from each wild type and transgenic type was transferred into 2-ml micro tubes containing 1 ml of incubation medium [potassium phosphate pH 5.8 (50 mM), tween

20 (1%), sucrose (2%), phosphinotricin (25 mg/l), and 2, 4- dichlorophenoxy acetic acid (0.1 mg l^{-1})] supplemented with 0, 25, 50, 100, 150, and 200 mg/l KCN. Three reaction micro tubes containing incubation media were used as blanks.Under 80 µmol m^{-2} s^{-1} light, the test samples were incubated for 16 hours at 25 ˚C. A volume of 100 µl of each samples was put into a fresh 1.5-ml reaction tube containing 0.5 ml of reagent-I which consist of [sodium nitroprusside (0.4 mM), sodium salicylate (0.21 M), trisodium citrate (0.085 M), and sodium tartrate (25 g/l)] after centrifugation at 13,000 rpm/5 min. Avolume of 500 µl of reagent-II which consist of [disodium dichloro isocyanurate (2.3 mM), and NaOH (0.75 M)] was added to the samples. After that, the reaction mixures were kept in the dark at 37 ˚C for 15 minutes before being incubated at room temperature. The absorbance at 655 nm of the test samples were measured and compared to ammonium chloride standards to determine the amount of ammonium ions present.

2.2.4 Extraction of protein and cyanase assays

100 mg fresh algal tissues from wild-type and transgenic *C. reinhardtii* cells were frozen in liquid nitrogen. After homogenising the frozen pellets, a volume of 500µl of extraction buffer were added. The homogenate was centrifuged twice for 20 minutes at 402 xg at 4 ˚C. The protein concentration in the supernatants was then measured according to [30]. Cyanase activity was evaluated according to the protocol described by [31]. The reaction mixture composed of [potassium phosphate buffer 50 mM (pH 7.7), 2 mM $NaHCO_3$ and 2 mM KCN]. The reaction was started by addition of protein extract and stopped by adition of equal volumes of Nessler's reagent after 1-10 min at 26 ˚C. A standard curve was created using ammonium chloride dilution series. The amount of ammonia released was estimated by measuring the optical density at 420 nm. The activity of cyanase was measured in µmol ammonia released/mg protein/min.

2.2.5 Extraction and determination of green pigments under cyanide stress

Green pigments, Cl. a, Cl. b, were extracted from wild-type and transgenic cells grown for 3 days in liquid TAP medium supplemented with different concentration of KCN (0.0, 25, 50, 100, 150, and 200 mg/l) by using acetone 80%. The mixture was centrifuged at 402 xg for 10 min after homogenyiation and vigorous shaking, absorbance of the supernatants was measured at 630, 647, 664 nm. Pigments contents were calculated as described by [32, 33].

2.2.6 Effeciency of both wild and transgenic genotypes to detoxifying cyanide at different pHs

In this experiment two sets of 250ml flasks containing 100ml sterile TAP medium supplemented with 25 mg/l of potassium cyanide and different pH values (4, 5, 6, 7, 8, 9, 10, and 11) were prepared one set inoculated with 2ml transgenic cells and the other with wild cells (inocula having $6*10^6$cells for each) and cultred for 24 h (The contact time), all the flasks were kept at 25 °C. At the end of incubation period, centrifugation was carried out and the clear solution was ready to determine the residual of potassium cyanide as previously mentioned to calculate the amount of cyanide removal.

Percentage of cyanide removal = $(C_0 - C_r) \times 100 / C_0$

Where C_0 is the initial concentration of cyanide (mg/l)

and C_r is the residual concentration of cyanide (mg/l).

2.2.7 Statistical analysis

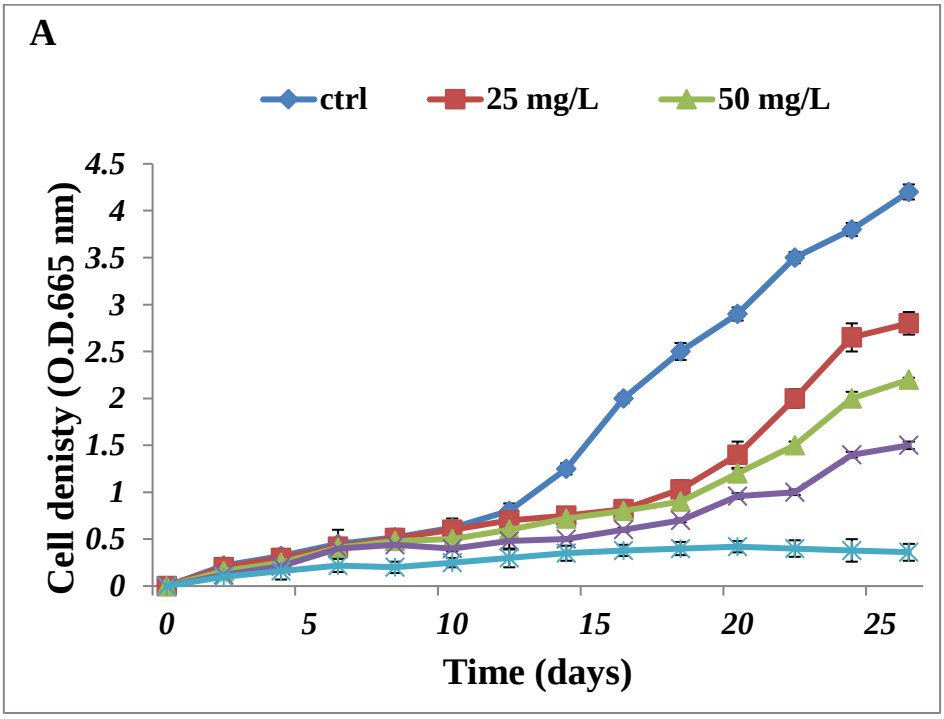
For each wild type and transgenic type the data was represented in figures as mean ± standard error (SE). The obtained data were analyzed statistically using SPSS to determine significant differences among the data. Difference was considered significant (*) when $P < 0.05$, highly significant (**) when $P < 0.01$ and very highly significant (***) when $P < 0.001$.

3 Results and discussion

3.1 Growth assays of both genotypes under cyanide stress

The results of this assay are shown in Fig.1 Both wild type and CYN transgenic lines showed a concentration-dependent inhibition of *Chlamydomonas reinhardtii* growth. The least reduction in algal growth was indicated following 25 mg^{-l} with speed reference to transgenic type this platoue was changeable to the high reduction in growth especially in case of wild type at 150 mg^{-l} and no growth at 200 mg^{-l} whereas, transgenic grow at 200 mg^{-l} with an obvious decrease due to natural rate of decay as the nutrient consumed. The obvious reduction in wild type growth in prescence of potassium cyanide may be attributed to the inefficient capacity of the endogenous cyanaide metabolizing enzymes to remove the applied cyanide from the growth [25, 34-35]. It is crucial to compare the current CYN growth assay resuls with previously

published CYN transgenic *A. thaliana* plant [36]. When cyanate concentration of 2.5 mM was applied to the foliar parts of the plants, a similar growth retardation effect was observed for wild type *Arabidopsis thaliana*. In addition, in wild-type *Arabidopsis* plants, 0.4 mM cyanate in the culture media completely inhibited root hair production. However, expression of the cyanobacterial cyanase enzyme in *Arabidopsis thaliana* plants improves plant resistance to exogenously cyanate up to 1.2 mM in both growth experiments. Indeed, the enhanced growth of CYN transgenic *C. reinhardtii* at higher cyanide concentrations (i.e., 100, 150, and 200 mg/l KCN) compared to wild-type cells suggests the usage of CYN transgenic *C. reinhardtii* as efficient bio-system for detoxification of cyanide from the environment especially, from the contaminated water sites.



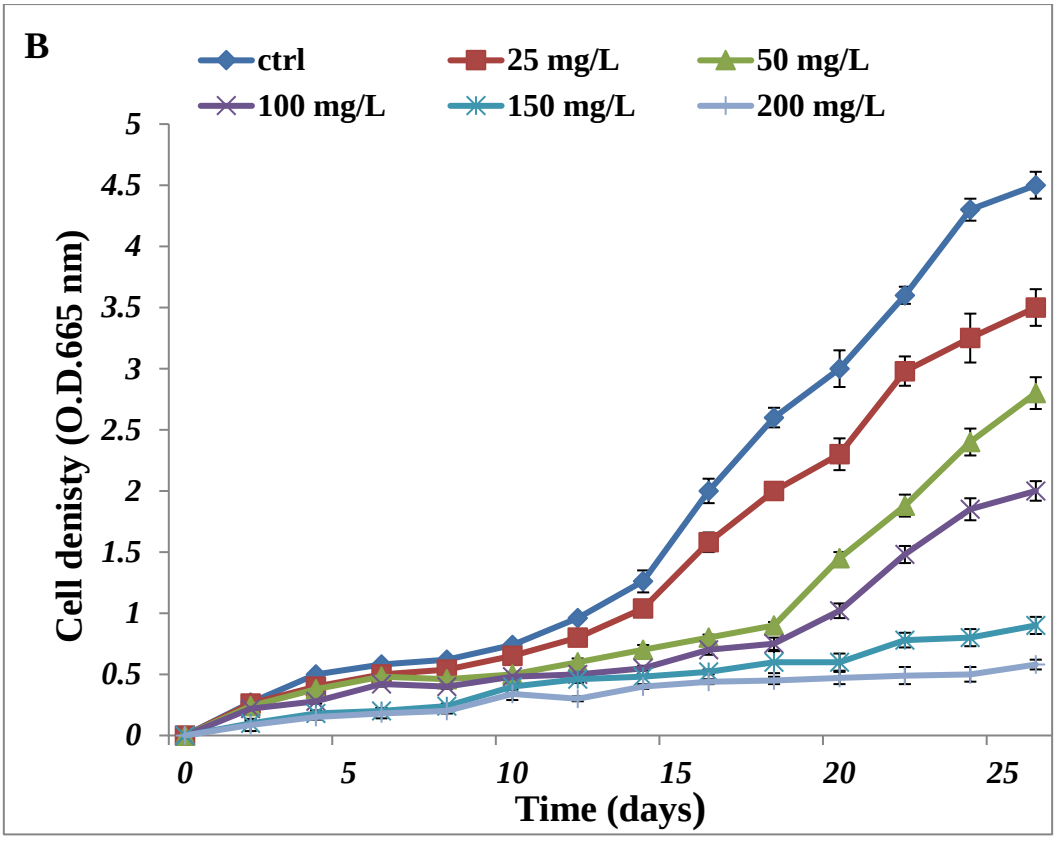


Fig.1: Growth of both (A) wild type and (B) transgenic type of *Chlamydomonas reinhardtii* in prescence of different concentrations of cyanide.

3.2 Efficiency of cyanide removal by both wild and transgenic *C.reinhardtii*.

Results in Table (1) revealed that transgenic type has promising efficiency to remove potassium cyanide when compared to wild type.

The rate of potassium cyanide removal reaches to 100% by transgenic type in presence of 25 mg/l after 24 hours, whereas in case of wild type the rate of removal reaches to 100% after 48 hours.

In case of application of 50 mg/l potassium cyanide it is obviously found that transgenic type completely remove this concentration after 36 hours, whereas in case of wild type amount of removal reaches to 35 mg/l from this concentration after 72 hours.

This mean that the contact time is effective parameter for removal i.e., increasing the rate of removal was parallel to increasing the contact time in case of transgenic type at the relatively low concentration (25 and 50 mg/l).

Results of relatively high concentration indicate that the wild type can resists the presence of cyanide up to 150 mg/l and couldn't be able to grow in presence of 200 mg/l, whereas, transgenic type has the ability to resists cyanide with very low degradation rate.

Table (1): Amount of cyanide removal (mg/l) at different concentrations and times (hours) by wild type and transgenic type of *C. reinhardtii.*

Time(hour) / **Conc.(mg/l)**	**Zero h**	**6 h**	**2 h**	**24 h**	**36 h**	**48 h**	**72 h**	**Total of amount removal (mg/l)**
	Wild type							
25	0.00 0.00	6.69 ±0.33	5.42 ±0.80	5.26 ±0.36	4.36 ±0.39	3.25 ±0.00	0.02 ±0.00	25
50	0.00 0.00	14.38 ±0.32	9.37 ±0.00	6.25 ±0.00	3.31 ±0.31	0.81 ±0.39	0.63 ±0.38	34.75
100	0.00 0.00	10.44 ±0.00	13.44 ±0.71	7.94 ±0.98	7.19 ±0.29	0.82 ±0.04	0.54 ±0.13	40.37
150	0.00 0.00	12.50 ±0.53	11.88 ±0.45	6.63 ±0.07	5.75 ±0.73	0.72 ±0.72	0.04 ±0.0	37.55
	Transgenic type							

Time(hour) / Conc.(mg/l)	Zero h	6 h	2 h	24 h	36 h	48 h	72 h	Total of amount removal (mg/l)
25	0.00 0.00	12.58 ±0.13	10.22 ±0.34	2.20 ±0.23	0 ±0.00	0 ±0.00	0 ±0.00	25
50	0.00 0.00	20.06 ±0.57	12.1 ±0.19	9.1 ±1.53	8.74 ±1.82	0 ±0.00	0 ±0.00	50
100	0.00 0.00	19.19 ±0.69	19.06 ±1.69	13.88 ±1.36	9.18 ±0.85	5.38 ±0.16	3.25 ±0.46	69.94
150	0.00 0.00	17.63 ±1.21	17.13 ±2.02	8.2 ±2.82	7.6 ±1.46	2.4 ±0.95	1.19 ±0.56	54.15
200	0.00 0.00	12.64 ±0.00	11.33 ±0.00	9.84 ±0.00	7.66 ±0.00	0.25 ±0.00	0.06 ±0.00	41.92

Data are the avarege of three replicates ± SE, ***p<0.001.

3.3 Ammonia released by transegenic *C. reinhardtii* under cyanide stress

Cyanase catalyzes the decomposition of cyanide into CO_2 and NH_3 in presence of bicarbonate. Therefore, the amount of ammonia released from wild type and transgenic *C. reinhardtii* lines in presence of variable concentrations of cyanide was measured. This assay relies on the inhibition of ammonia fixation/re-fixation in the GS2 (Glutamine synthase2)/Fd-GOGAT (Ferredoxin-dependent Glutamine Oxoglutarate Amino Transferase) cycle in the presence of phosphinotricin. Fresh *C.*

reinhardtii wild type and transgenic *Chlamydomonas reinardtii* cells were incubated in medium contaning different concentration of KCN (i.e., 0.0, 25, 50, 150, and 200 mg/l) and 2 mM $NaHCO_3$ as described in materials and methods section. Absence of KCN, in control and NH_3 release was not particularly evident in both transgenic and wild type control samples. A gradual decrease in the amount of NH_3 was observed for wild type *C. reinhardtii* by increasing KCN concentrations (Inverse relationship). On contrary, significant KCN concentration dependent ($p \leq 0.001$) increases in the amount of NH_3 released was observed for all the two CYN transgenic lines compared to wild type controls (Direct relationship). Again, line Cr.CYN-2 was superior when compared to Cr.CYN-1 Fig.2. The reduction of ammonia release observed for wild type control may be attributed to the limited capacity of the endogenous cyanase to overcome the excess cyanide applied to the reaction mixture [35]. Induction of the cyanase gene transcription in microorganisms can be accomplished with the use of exogenous cyanide [37]. However, induction of cyanase gene transcription is almost absent in plants because endogenous cyanase gene transcription was only observed to be up regulated under salt stress [34, 35].

The results of ammonia release experiment show that CYN transgenic *C.reinhardtii* can produce more ammonia from exogenously applied cyanide, demonstrating the functioning of the cyanobacterial cyanase enzyme in *chlamydomonas reinhardtii*. This emphasises the importance of cyanide detoxification using CYN transgenic lines. Also, the result indicated that the nitrogen from ammonia accumulated directly as an intermediate product before nitrate instead of being completely nitrified [38]. Despite this, ammonia accumulation was affected by decreasing cyanide degradation because ammonia inhibits boi-oxidation due to the participation of microbial enzymes in cyanide degradation, nitrification in the treatment system [39, 40]. However, there was a changing concentration of ammonia in the medium; the high ammonia concentration could inhibit cyanide biodegradation because organisms prefer ammonia rather than cyanide as a nitrogen source, thus reducing cyanide degradation by microbial species [41].

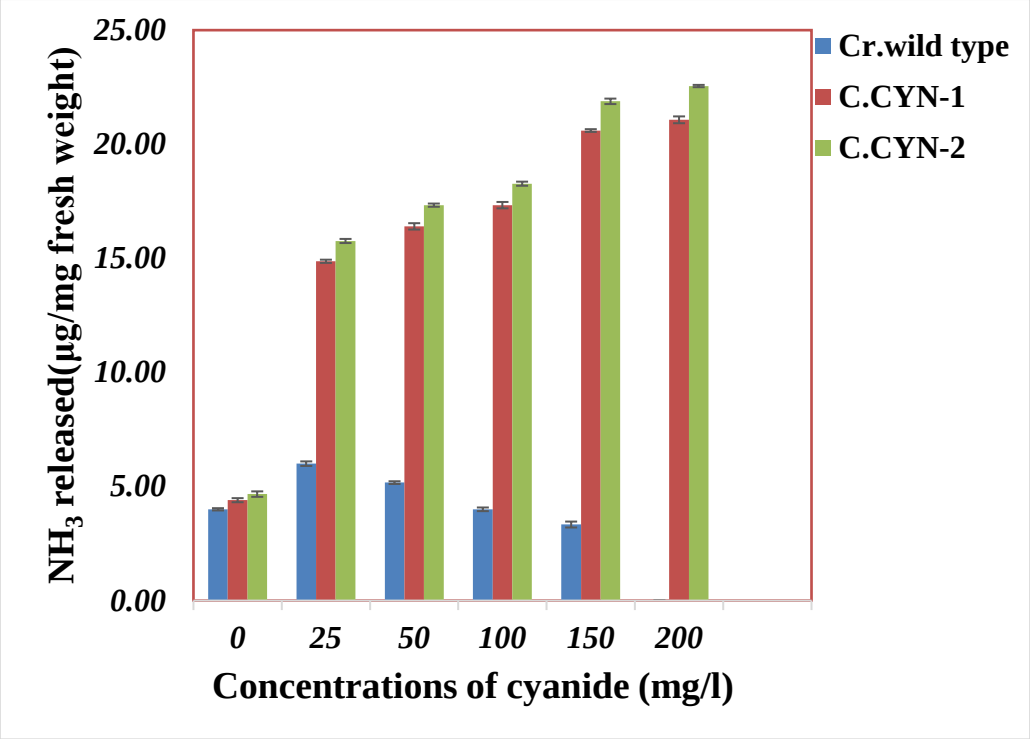


Fig.2: Amount of ammonia released from CYN transgenic and wild type *C. reinhardtii* under differernt concentrations of KCN.

3.4 Cyanase activity

Cyanase activity *in vitro* was also tested in extraction of protein of both wild and transgenic types Table (2). Results revealed that cyanase reachs its maximum activity following the application of 100 mg^{-l} KCN. It reached eight folds more than the corresponding activity in wild extract. Moreover, in general as the concentration increased the activities in both wild type and transgenic type increased up to 50 mg^{-l} followed by sharp decline in the activity of wild extract especially, in presence of relatively high concentrations of cyanide (100 and 150 mg/l). On the other hand, activity in transgenic type showed an obvious increase up to high concentration. Endogenous cyanases and other nitrogen-assimilating enzymes may be responsible for the background activity in wild control [42,43]. Consequently, it can be concluded that the prokaryotic cyanase is active in transgenic *C. reinhardtii* lines and help the species to tolerate the toxicity of cyanide.

Table (2): Cyanase activity as µ mol ammonia released / mg protein/ 1 min

Treatment mg/l	µ mol NH_4/mg protein/ 1 min	
	Wild type	Transgenic type
0.00	2.15 ±0.11	2.30 ±0.15
25	5.16 ±0.15	9.11 ±0.25
50	6.63 ±0.10	15.60 ±0.0
100	2.30 ±0.00	18.70 ±1.0
150	1.16 ±0.00	6.11 ±0.32

Data are the avarege of three replicates ± SE

3.5 Effect of cyanide on chlorophyll a and b of wild and transgenic *C. reinhardtii* at different time.

Fig.3 and Fig.4 showed that most concentrations of cyanide lead to subsequent inhibition in chlorophyll a and b as compared with untreated sample. The least reduction was observed at relatively low concentrations, whereas at higher concentrations, the rate of reduction of chlorophyll a and b content was pronounced, with an obvious dropping of chlorophyll in both types. In the case of wild type, application of higher concentration (200 mg/l) resulted in complete delitorus with the cells and, finally, complete death. Also, it could be observed that biosynthesis of chlorophyll a as indicated by it's content was increased with increasing the time in case of transgenic especially, at 25 mg/l but still lower than untreated transgenic one. The previous behaviour was countered at 25 mg/l treated wild as the time increased. The content of chlorophyll a and b increased with maximum production after 72 h. A similar result of reduction was indicated in wild sample as increasing the cyanide concentration and time. The percentage of reduction in chlorophyll a in wild type was 75.82% but in case of transgenic type was 37.3%. On the other hand, the percentage of reduction in chlorophyll b in wild type was 81.36% but in case of transgenic type the percentage of reduction in chlorophyll b reached to 35.75%. The previous reductions were in concomitant with [44] who found that heavy metal (Cu) reduce and

inhibits pigment production in *Scenedesmus quadricauda* and similar results was also obtain in case of *Scenedesmus obliquus* [45]. Also, pigment reduction was reported for *Chlorella vulgaris* exposed to KCN [46]. Also, similar results were obtained in case of *Chlorella vulgaris* exposed to Cr [47] and also in case of exposed to waste water effluents [48, 49, 50]. The prior decline could be explained by the heavy metal counteracting Fe and Mg absorption and so inhibiting chlorophyll production [51]. In our study the decrease in Chl. a/Chl. b for wild type *C. reinhardtii* could be due to cyanide-dependent inhibition of enzymes involved in numerous metabolic processes related to pigment production and viability [52, 53]. These findings suggested that wild-type Chl. b is more sensitive than transgenic Chl.b. As a result of the oxidation of the methyl group on the ring II, some Chl.a can be changed to Chl.b [54]. These results are matched with [55] who found that the increase of Chlorophyll *a*/*b* in *Chlorella vulgaris* under 0.5 Cd mg/l. The mitochondrial cytochrome C oxidase that is actively involved in the respiratory electron transport chain was reported to be inhibited by cyanide and its oxidative product cyanate [56]. Other enzymes such as ribulose- 1,5 bisphosphate carboxylase/oxygenase [57] and enzymes involved in reactive oxygen scavenging [58] are proved to be inhibited by cyanide. Additiionally, cyanide and cyanate are known to inhibit biological processes including respiration, carbon and nitrate assimilation by binding to metalloenzymes [52, 59]. Transgenic *Arabidopsis thaliana* plants overexpressing the cyanobacterial cyanase enzyme [36] or overexpression the bacterial cyanidase enzyme [60] showed significant increase in chlorophyll a, chlorophyll b, and carotenoids contents compared to control plants under cyanate or cyanide stress respectively. The observed result in this assay indicates that KCN induced loss of pigmentation was alleviated through over expression of the cyanobacterial enzyme cyanase in *C. reinhardtiin* cells.

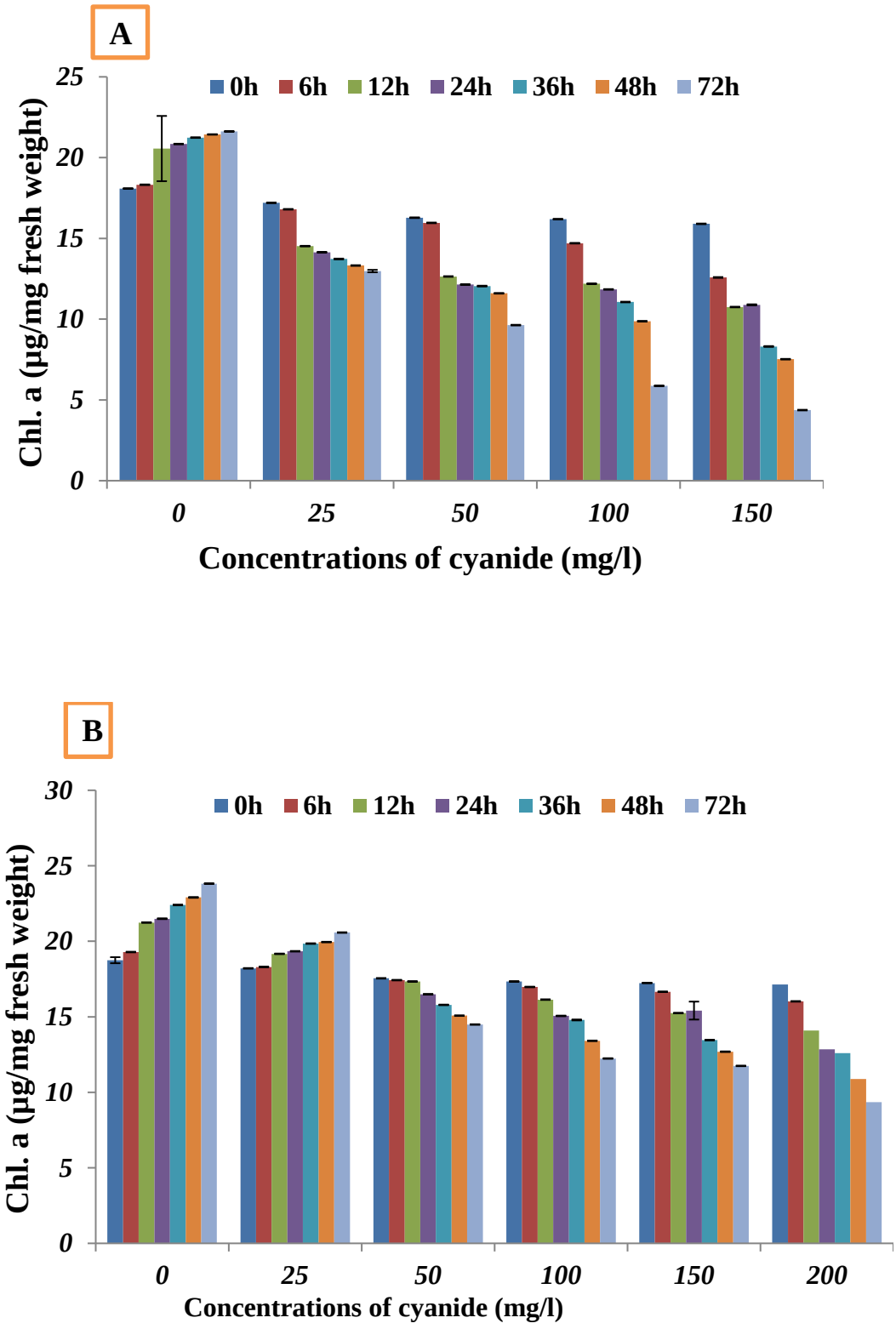


Fig.3: Effect of different concentrations of cyanide on chlorophyll a of both (A) wild type and (B) transgenic type of *C. reinhardtii* at different times.

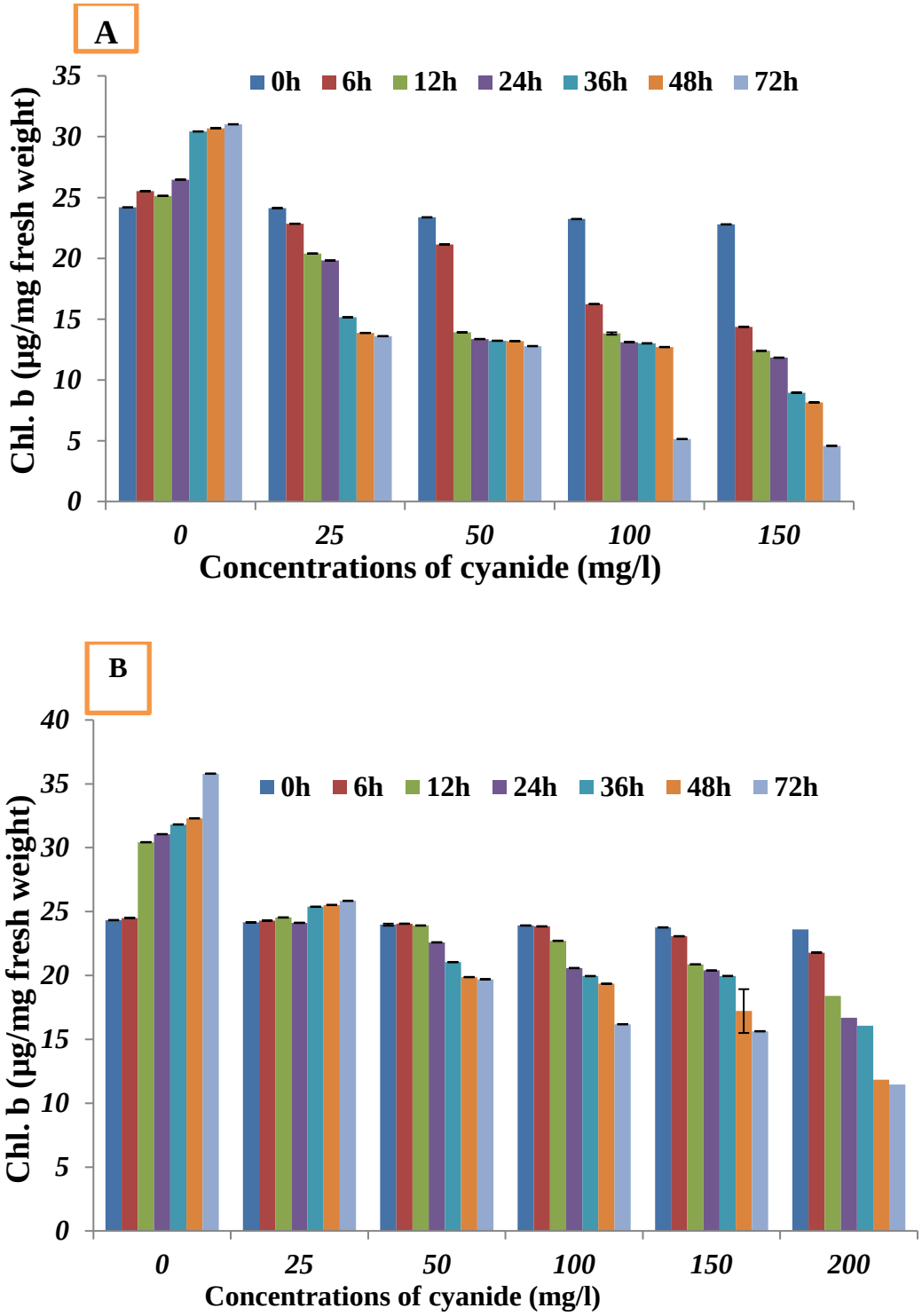


Fig.4: Effect of different concentrations of cyanide on chlorophyll b of both (A) wild type and (B) transgenic type of *C. reinhardtii* at different times.

3.6 Effeciency of both wild and transgenic genotypesto detoxifying cyanide at different pHs

Fig.5 revealed that the rate of removal gave subsequent increasing as the pH increased from pH 5 to 11. The maximum recorded following the application of pH 10 (95.09%) then decreased to (90.88%) at pH 11. In wild type the rate of removal reaches the maximum level at pH 9 (45.96%) less than transgenic at pH 9 (92.96%). Moreover, the subsequent removal of cyanide by transgenic cells resulted in two folds increase more than the corresponding values in case of wild type. The previous results are in concomitant with the explanation that, microbial activity can reduce cyanide to ammonia, which oxidises to nitrate under aerobic circumstances. With cyanide concentrations up to 200 parts per million, this technique has proven to be effective [61]. He also, demonstrated that the best conditions for cyanide breakdown were 34 $^{\circ}$C, pH 10.3, and a glucose content of 0.44 (g/l). Furthermore, cyanide degradation reached a maximum level at 96 percent during the exponential period. *Burkholderia cepacia* can remove cyanide in a pH range of 8 to 10, with the highest cyanide removal (1.85 mg CN. $h^{-1)}$ occurring at pH 10 [26]. (Under alkaline conditions (pH 9.2–10.7), Cyanide was metabolised via a cyanide hydratase and amidase route [62]. At pH of 9.5, cyanide degradation by the strain *Pseudomonas pseudoalcaligenes* CECT5344 may therefore provide.

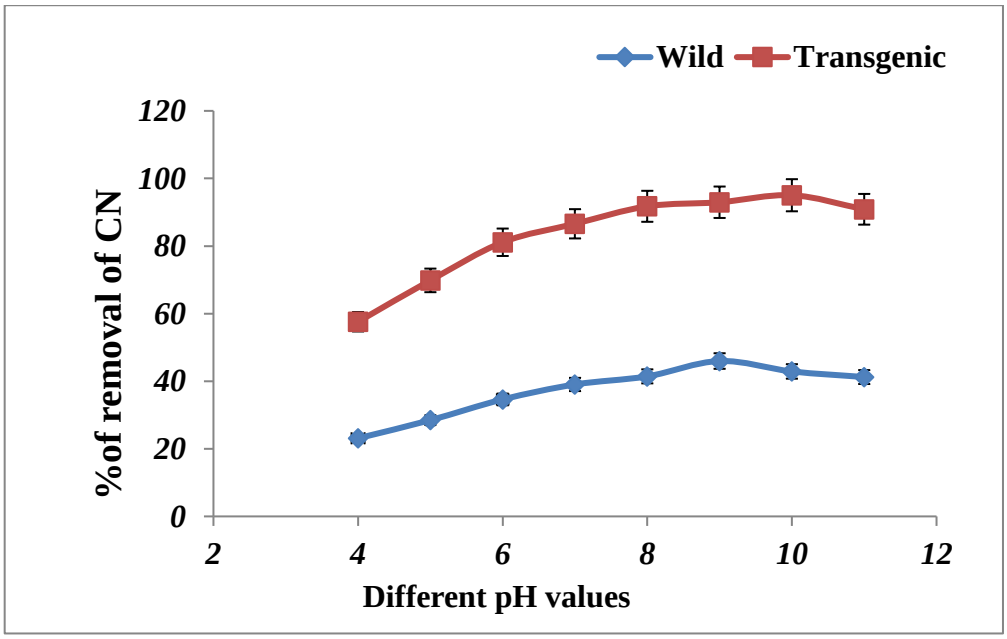


Fig.5: Percentage of cyanide removal by wild and transgenic type of *C.reinhardtii* at different pH values.

4. Conclusion

The results have indicated a novel mechanism for bioremediation of cyanide toxicity from water using transgenic *Chlamydomonas reinharditii* alga. Such results confirmed that CYN gene reduce the toxicity of cyanide and this represented in the measured biochemical parameters. Cyanide depletion assays and algal growth showed a significant increase in transgenic type to resist cyanide stress as compared to wild type. The presence of CYN gene has induced a protection response to the biosynthesis of green pigments. Visible phytotoxic symptoms due to cyanide application was only observed in wild type alga. The development of phycoremediation system for the degradation of toxic contaminants in agricultural fields could benefit from transgenic algae detoxifying cyanide. The current results could be applied to other crops in programs aimed at increasing pollution tolerance and limiting contamination in the food supply chain. As a result, the transgenic *C. reinhardtii* strain has shown promise in a wide range of cyanide detoxifying environments.

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الملخص العربي

كفاءة طحلب الكلاميدوموناس المعدل وراثيا في ازالة سمية مركب السيانيد

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الملخص العربي

تزايدت في الآونه الأخيره سرعة تطوير تقنيات جديدة لمعالجة النفايات الصناعية والخطرة،وذلك من خلال التركيز بشكل خاص على استخدام أنظمة المعالجة البيولوجية،حيث نجحت عمليات المعالجة الميكروبية (الهوائية واللاهوائية) في تحليل و / أو إزالة المركبات العضوية وغير العضوية والعناصر الثقيلة. ويعتبر السيانيد من أكثر هذه المركبات استخداما، وهي من المواد الكيميائية السامة المعروفة التي يتم إنتاجها من خلال الأنشطة والصناعات البشرية التي تستخدم في ترشيح المعادن الخام والطلاء المعدني بالكهرباء وإنتاج الصلب والبلاستيك والألياف الصناعية.

وأجريت الدراسة بنموالسلالة المعدلة وراثيا من طحلب الكلاميدوموناس والغير معدلة تحت تركيزات مختلفة من مركب السيانيد (25 ، 50 ، 100 ، 150 و 200ملليجرام /لتر) أظهرت الدراسة قدرة السلالات المعدلة وراثياعلي النمو في التركيزات العالية مقارنة بالسلالة الغير معدلة خاصة عند تركيزي 150 وتركيز 200 ملليجرام/لتر.

وعند معاملة الطحالب (المعدلة وراثيا والغير معدلة) بتركيزات مختلفة من السيانيد (25 ، 50 ، 100 ، 150 و200ملليجرام /لتر) أظهرت السلالات المعدلة وراثيا كفاءة عالية في ازالة السيانيد، مقارنة بالسلالة الغير معدلة . عند أقل تركيز من السيانيد 25 ملليجرام بلغت نسبة ازالة السانيد 100% بعد 24 ساعة للكائن المعدل وراثيا بينما الكائن الغير معدل وراثيا بعد 48 ساعة.عند التعرض للتركيز العالي من السيانيد 200 ملليجرام لم يستطع الكائن الغير معدل وراثيا النمو

كما أظهرت الدراسة قدرة السلالات المعدلة وراثيا إنتاجا عاليا من الأمونيا بالاضافة الي انخفاض في محتوى الأصباغ للكائن الغير معدل وراثيا مقارنة بالكائن المعدل وراثيا.حيث بلغت نسبة اختزال كلوروفيل .أ .37.3% في السلالة المحورة وراثيا مقارنة ب 75.82% في السلالة الغير محورة وراثيا.وأيضا قد بلغت نسبة اختزال كلوروفيل. ب 35.75 % في السلالة المحورة وراثيا مقارنة ب 75.82% في السلالة الغير محورة وراثيا.