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Biosorption Potential of the Microchlorophyte *Chlorella vulgaris* for Some Pesticides

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Abstract

Nowadays, pollution of either surface or ground water with pesticides is considered as one of the greatest challenges facing Humanity and being a national consideration in Egypt. Agricultural activities are the point source of pesticides that polluting water bodies. The present study investigated the potentiality of *Chlorella vulgaris* for bioremoval of pesticides mixture of 0.1 mg/mL for each component (Atrazine, Molinate, Simazine, Isoproturon, Propanil, Carbofuran, Dimethoate, Pendimethalin, Metoalcholar, Pyriproxin) either as free cells or immobilized in alginate. Two main experiments were conducted including short- term study having 60 min contact time using fresh free and lyophilized cells and other long-term study having five days incubation period using free and immobilized cells. In the short-term study, the presence of living cells led to bioremoval percentage ranged from 86 to 89 and the lyophilized algal biomass achieved bioremoval ranged from 96% to 99%. In long-term study, the presence of growing algae resulted in pesticides bioremoval ranged from 87% to 96.5%. The main mechanism behind the removal of pesticides in water phase is proposed to be biosorption onto the algal cells. This conclusion is based on the short duration required for removal to occur. The obtained results encourage using microalgae in bioremediation of pesticides polluted water.

Keywords: Biosorption; *Chlorella vulgaris*; Algal immobilization; Pesticides; Bioremoval efficiency; LC-MS/MS

Introduction

Recently, application of pesticides is known in everywhere all over the world resulting in exposing the general population to low concentrations of pesticides used in agriculture as herbicide, insecticides and fungicides for controlling plant pests as well as contamination of air, water and foods [1,2]. Pesticides contamination of water has been well documented worldwide to be considered as a potential risk for the ecosystem. Pesticide residues are frequently present in the aquatic environments according to surface runoff, leaching from surface pesticides applications and via industrial activates and/or domestic sewage as founded by Miliadis, Tikoo and Priyadarshani [3-5]. This is why we are in an urgent need for developing some efferent bioprocesses for remediation of pesticides pollutants. Biosorption process is one of the bioremediation mechanisms which is favorable, using living microorganisms as fungi, microalgae as well as bacteria for recovery process that have low costs as suggested by Naturvårsverket [6,7].

In the near future, water reusing will become very important in densely populated arid areas where there is an increasing demand to supply water from limited supplies. Human well-being in a future world will depend mostly upon this sustainable resource and the characterization of emerging contaminants will become important for ecological and human health risk assessments and commodity valuation of water resources [8,9]. Egypt characterized with developing agricultural activities accounted 28% of the total national income, and nearly half of the country's work force is dependent on the agricultural subsector for its livelihood.

There are anaggravating chemical environmental contamination by attributed to using organo-chlorinated pesticides, herbicides, fungicides as well as insecticides that are anticipated along the Nile Delta, which is referred to as "Green Lungs of Egypt" [10]. Moreover, chemical industries in Egypt is one of the main sources of hazardous wastewater. Barakat suggested that, water pollution is exacerbated by agricultural pesticides, raw sewage, and urban and industrial effluents [11]. Consequently, remediation of pesticides from water bodies as well as ground water is very urgent, especially bioremediation by microalgae.

Chemical properties of the pesticide such as molecular weight, functional groups and toxicity affect the metabolic degradation of it [5]. Algae appear to be more able to metabolize organic compounds with low molecular weights than larger molecules [12-14].

The main objective of this study is to investigate the capability of the microalga *C. vulgaris* either free or immobilized cells for bioremoval of ten pesticides mixture.

Materials and Methods

Microalgae

Fresh water *Chlorella vulgaris* was isolated from water sample from river Nile. Culture purification was according to Andersen [15] and the alga was identified according to Philipose [16]. *Chlorella vulgaris* was grown in axenic cultures at 27 ± 2°C under continuous illumination 3600 lux in 500 ml Erlenmeyer flasks, containing 200 ml BG11 medium for 5 days incubation period in an IlluminatedMemmert incubator (Memmert GmbH+Co. KG, Germany) [17]. The starting inoculum size of *C. vulgaris* was 10% (v/v) taken from 5 day-old culture, supplemented as biomass pellet

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after centrifugation (3000 g, 15 min, Bench-top - TD5B, Germany). Lyophilized algal biomass was prepared from 5 day-old culture pellets that washed once with distilled water and lyophilized in a freeze dryer for 24 h. The lyophilized biomass was stored under dark conditions at room temperature, while living biomass was produced under the same growth conditions.

Selected pesticides

Custom standard mixture (Atrazine, Molinate, Simazine, Isoproturon, Propanil, Carbofuran, Dimethoate, Pendimethalin, Metoalcholar, Pyriproxin) 0. 1 mg/mL for each in methanol was purchased from Accustandard Inc., USA. The standard was obtained from The Reference Laboratory for Drinking Water, Cairo, Egypt. Standard solution containing the 10 microcontaminants in methanolic solution was added to each flask (final water or medium volume of 0. 1 L) to obtain a final concentration of 2 µg L^{-1} and 10 µg L^{-1}. The concentration 10 µg L^{-1} waskeptin high concentration level for further detection of pesticides in agricultural surface water following a runoff or spray drift events [18,19].

Experimental set-up

The short-term study: An initial concentration of 2.0 µg L^{-1} and 10 µg L^{-1} was obtained by adding the pesticide mix to sterile Milli Q water. The experiments included Lyophilizedalgal biomass, living algalbiomass and a control without any biomass, with three replicates per experiment. The amount of biomass (living biomass or lyophilized biomass) added to each replicate corresponded to 10% (v/v) taken from 5 day-old culture. There were three replicates per treatment and the total volume of each culture was 100 ml. The treatments were stirred on anorbital shaker at a speed of 380 rpm for 1 h at room temperature (Thermo Scientific™ MaxQ™ 4450 Benchtop Orbital Shakers, USA). After one hour, the biomass was removed from the aqueous phase by centrifugation and the samples were stored in the freezer at -20°C until analysis.

The long-term study: Final pesticides concentrations of 2.0 µg L^{-1} and 10 µg L^{-1} was obtained by supplementing the pesticides mixture to sterile BG11 with inoculum volume of 10% (v/v) of a five-day old culture with total volume of 100 ml. The experiments were kept under the growing condition described above for 5 days. After the experiment the biomass was removed by centrifugation and samples of the aqueous phase were taken and stored in the freezer until analysis.

Preparation of immobilized algae-alginate beads: After 7 days culturing, algal cells at their log phase were harvested by centrifugation at 5000 rpm for 10 min at 48°C, the pellet was washed and re-suspended in sterilized deionized water (20 ml). This concentrated algal suspension was then mixed with 3% (w/v) sodium alginate solution in 1:3 volume ratios to yield a mixture of algal-alginate suspension which was dropped into calcium chloride solution (2.5%) using magnetic stirrer (CORNING BC620D, USA) to form uniform algal beads. The algal beads were left in $CaCl_2$ solution for 12 h forhardening. Blank alginate beads were prepared in the same way as the algal beads without adding algal cell suspension.

Chromatographic analyses: Samples (50 ml) from the aqueous solution were sent to The Reference Laboratory for Drinking Water, Cairo, Egypt for chromatographic analyses. Reference method EPA 536, were used to conduct the pesticides analysis, which is based on a combination of liquid chromatography (LC) and mass-spectroscopy (MS) specifically called LC-MS/MS (tandem-MS) [20]. Tandem-MS (Xevo-TQ-S, Waters Corporation, Milford, MA, USA) provides low detection limits and very high security, which means that more substances can be tracked at lower level [21].

Results

Short-term study

As illustrated from Figure 1, the highest pesticides bioremoval activity of *Chlorella vulgaris* living cells was recorded to the herbicide atrazine (0.213 µg/l) with initial concertation 2 µg/l, while the herbicide isoproturon recoded the minimum biosorption (0.291 µg/l). Starting with 10 µg/l pesticide mixture, the maximum absorption was documented to the herbicide molinate and the minimum to pendimethalin (1.112 µg/l and 1.687 µg/l respectively).

Concerning 2 µg/l initial concentration, the highestlyophilized *C. vulgaris* biosorption activity was documented for the herbicide isoproturon, while the minimum activity was confirmed to herbicide molinate. Initial concentration 10 µg/l induced maximum bioremoval of the herbicidecarbofuran (0.2178 µg/l), whereas the minimum activity was confirmed to atrazine (0.3712 µg/l) as illustrated in Figure 2.

Starting with 2 µg/l pesticide mixture, living *C. vulgaris* exhibited good biosorption efficiency ranged from (85. 60% to 88. 15%) which was documented for Atrazineand Isoproturn respectively. Concerninginitial conc 10 µg/l *C. vulgaris* lyophilized cells showed high biosorption efficiency ranged from (83.13% to 88.88%) for Molinateand Pendimethalin respectively (Figure 3).

Starting with pesticide mixture concentration (2 µg/l) lyophilized *Chlorella vulgaris* exhibited good biosorption efficiency ranged from (98.6% to 99.36%) which was documented for Isoproturn and molinate respectively. Concerning initial concentration 10 µg/l *C. vulgaris* lyophilized cells showed high biosorption efficiency ranged from (96.29% to 97.822%) for carbofuran and atrazine respectively as indicated from Figure 4.

Long-term study

Long-term study by living *C. vulgaris* biomass: Long term experiment with living *C. vulgaris* biomass (Figure 5) reviled that, bioremoval activity reached the maximum level (0.065 µg/l) with the herbicide simazine, while the minimum bioremoval activity was indicated to pendimethalin (0.243 µg/l) with the initial pesticides concentration 2 µg/l. Although beginning with 10 µg/l, the maximum

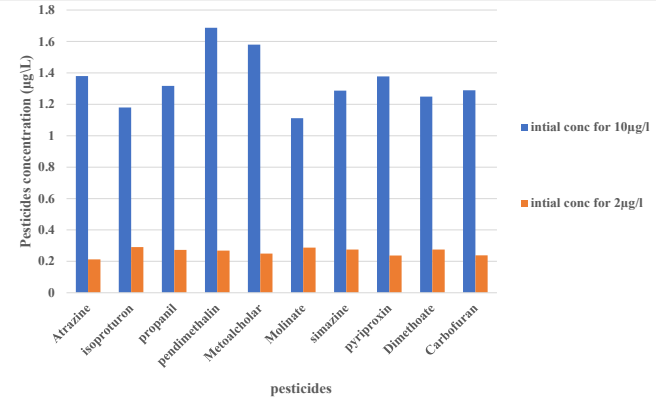

Figure 1: Graph showing amount of trehalose (mg/µl) treated with aqueous extract in adult *C. chinensis*. All values are mean ± SE of six replicates and significant at $p \leq 0.05$ level of significance.

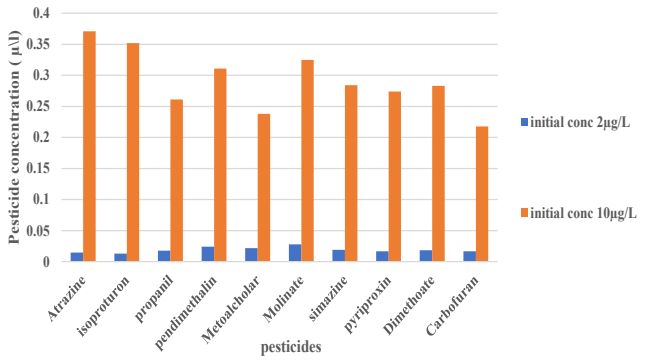

Figure 2: Residual pesticides concentration after biosorption by lyophilized cells of *Chlorella vulgaris* starting with two initial concentrations (2 µg/l and 10 µg/l) after one hour contact time.

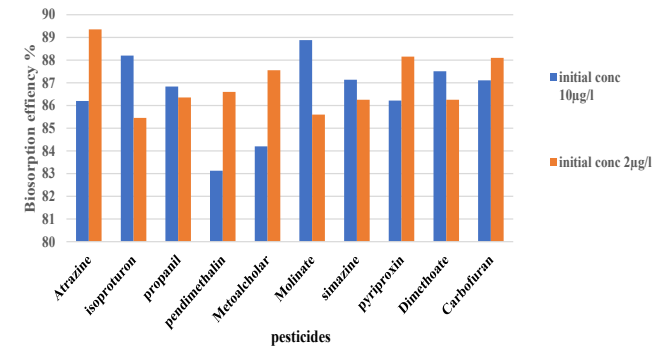

Figure 3: Biosorption efficiency of living cells of *Chlorella vulgaris* for pesticide mixture starting with two initial concentrations (2 µg/l and 10 µg/l) after one hour contact time.

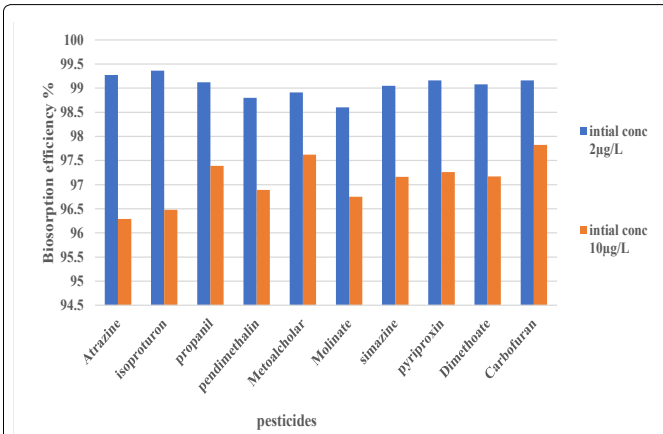

Figure 4: Biosorption efficiency of lyophilized cells of *Chlorella vulgaris* for pesticide mixture starting with two initial concentrations (2 µg/l and 10 µg/l) after one hour contact time.

biosorption activity (0.43 µg/l) was restricted to simazine and the minimum activity (1.186 µg/l) was illustrated to propanil.

Long term study by *C. vulgaris*-alginate beads: Immobilization of *C. vulgaris* cells alginate showed pronounced pesticide bioremoval activity with the two initial concentrations 10 µg/l and 20 µg/l through 5 days incubation period. Figure 6 illustrated maximum (0.157 µg/l) and minimum (1.026 µg/l) bioremoval activity of the two pesticides pendimethalin and propanil respectively for 10 µg/l as initial concentration whereas (0. 003 µg/l and 1.25 µg/l) represented the maximum and minimum bioremoval activity recorded for carbofuran and isoproturon, respectively starting with 20 µg/l.

Long term experiment with living *C. vulgaris* biomass (Figure 7) demonstrated that, biodegradation efficiency reached its maximum level (96.75%) with the herbicide simazine, while the minimum absorption activity was restricted to pendimethalin (87.85%) with the initial pesticides concentration 2 µg/l. Although beginning with 10 µg/l, the maximum biosorption efficiency (95.7%) was recorded to simazine and the minimum activity (88.14%) was illustrated to propanil.

In respect to biosorption potentiality and biodegradation, immobilization of *C. vulgaris* cells in alginate maintained distinct pesticide biosorption potentiality with the two initial concentrations 10 µg/l and 20 µg/l for 5 days incubation period. Figure 8 illustrated maximum (98.43%) and minimum (89.74%) biosorption activity of the two pesticides pendimethalin and propanil respectively for 10 µg/l as initial concentration whereas (87.5% to 99.97%) represented the maximum and minimum biosorption efficiency recorded for carbofuran and isoproturon, respectively starting with 20 µg/l.

Discussion

Recently, the spreading use of pesticides in the exhaustive agricultural activities and the modern daily life as well resulted in urgent environmental complications needing non-conventional treatment strategies as using of microalge for bioremediation processes. Present study indicated that, short-term study using both living and lyophilized biomass of *C. vulgaris* achieved high removal percentages as illustrated in Figures 1-4. The bioremoval of these pesticides over a period of 60 minutes suggested that the probable mechanism as biosorption according to the finds of Komárek, whereas, in Long-term study, there was sufficient time for some mentalizations processes occurred and the algae may either have biosorbed, metabolized or facilitated the degradation of pesticides, or it can be attributed to a combination of all these mechanisms [22]. Exhaustive agricultural activates which depends on using agrochemicals as fertilizers and pesticides, also they increase the global food production but also the at the same time contaminate the environment extensively as suggested by Cáceres, Singh and Walker [23,24].

Algal cell size, density, morphology and physiological activates can be attributed to biosorption and removal of pesticide. Microalgae is characterized by processing high surface area to biovolume ratio which provide high potential for sorption and the following inter action with pesticides. microalgae can utilize pesticide at the nontoxic levels. This was explained by Butler who reported that some macroalgal species (*Chlorella, Monoraphid, Actinastrum, Scenedemu, Nitzschia*) had the ability to degrade herbicides as following:1 ppm of carbaryl and diazinon, and 0.01 ppm of methoxychlor and 2,4-D [25]. Microgreen algae *C. vulgaris* and *Scenedesmus bijugatus* could metabolize organophosphorus insecticides (mon ocrotophos and quinalphos) while some cyanobacteria (*Synechococcus elongatus, Phormidium tenue* and *Nostoc linckia*) could metabolized these pesticides in the range of 5 to 50 ppm via 30 days as demonstrated by Megharaj [26]. *Anabaena* sp. and *Aulosira fertilissima* stored DDT, fenitrothion and chlorpyrifos in their cells as indicated by Lal, who reported that *Anabaena* sp. Absorbed 1568 ppm DDT, 3467 ppm fenitrothion and 6779 ppm chlorpyrifos, while *A. fertilissima* stored 1429 ppm DDT, 6651 ppm fenitrothion and 3971 ppm chlorpyrifos; where as these cyanobacteria species could metabolize DDT to DDD and DDE [27].

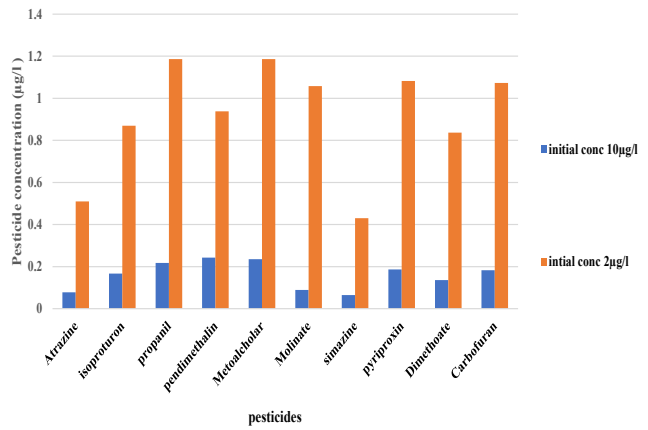

Figure 5: Residual pesticides concentration after biosorption by living cells of *C. vulgaris* starting with two initial concentrations (2 µg/l and 10 µg/l) after incubation period of 5 days.

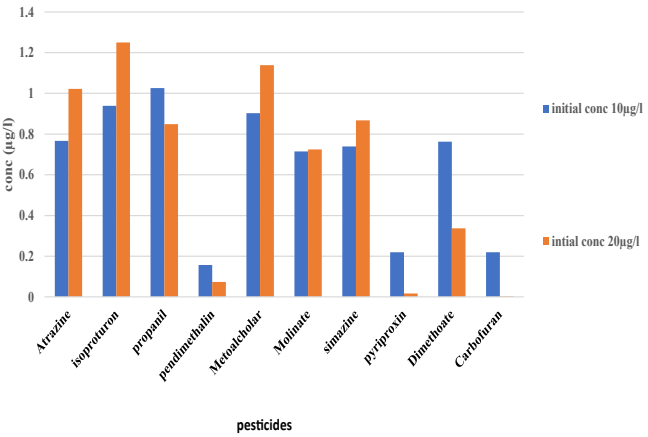

Figure 6: Residual pesticides concentration after biosorption by *C. vulgaris*-alginate beads starting with two initial concentrations (10 µg/l and 20 µg/l) after incubation period of 5 days.

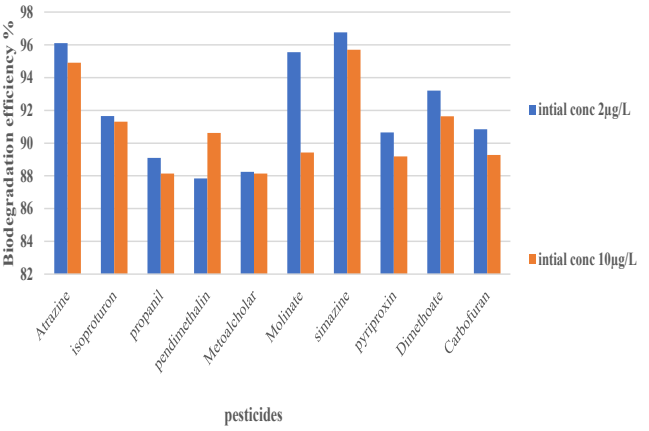

Figure 7: Biodegradation efficiency of pesticides for *Chlorella vulgaris* living cells starting with different initial concentrations (2 µg/L and 10 µg/L) after incubation period of 5 days.

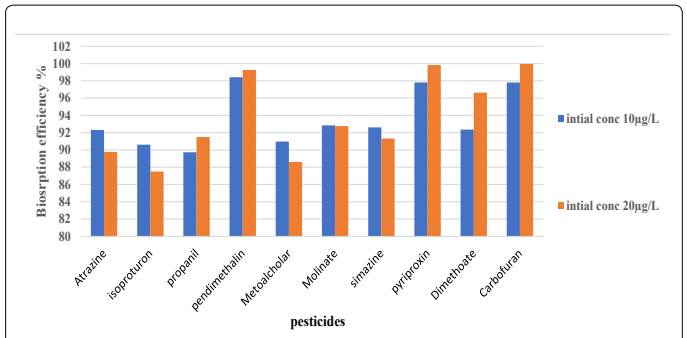

Figure 8: Biodegradation potential of pesticides for *C. vulgaris* - alginate beads starting with two initial concentrations (10 µg/L and 20 µg/L) after incubation period of 5 day.

Megharaj, indicated that greenalgae (*C. vulgaris and S. bijugatus*) and cyanobacteria (*N. linckia, Nostoc muscorum, Oscillatoria animalis* and *Phormidium foveolarum*) could metabolized the organophosphorus insecticide methyl parathion, and as a phosphorus source [28]. After 30 days of incubation, *O. animalis* and *P. foveolarum* totally biosorbed 20 µg mL^{-1} methyl parathion as well as its hydrolysis product PNP, whereas *N. muscorum* could oxidize the nitro group of PNP to nitrite via fifteen days. Each of *N. muscorum* and *A. fertilissima* could utilize each of the following monocrotophos (100 ppm), malathion (75 ppm), dichlorovos (50 ppm) and phosphomidon (25 ppm) as suggested by Subramanian [29]. Mode of nutrition could interfere with the ability of the pesticide detoxification of some algae. *C. vulgaris* with stand poisonous concentrations of carbofuran when cultivated ixotrophicallyon glucose or acetate as external cabon sources [30].

Concerning atrazine, Tang found that green algae as *Chlamydomonas sp.*, *Chlorella sp.*, *Pediastrum sp.*, and *S. quadricauda* could biosorbe more atrazine compared with diatoms (*Cyclotella gamma, Cyclotella meneghiniana, Synedra acus* and *Synedra radians*) [31]. The differential selectivity of algae species to atrazine can be attributed to their morphology, Cytology, physiology, phylogenetic, studied atrazine toxicity, accumulation and biodegradation in the microchlorophyte *Chlamydomonas mexicana* which exhibited accumulation and biodegradation potential resulting in 14-36% atrazine degradation at 10-100 µg L^{-1}. With high concentrations, reduction in total fatty acids (from 102 to 75 mg/g^{-1}) and increasing the unsaturated fatty acid content was observed, while carbohydrate content increased gradually with increasing atrazine concentrations up to 15% [32,33].

Living or immobilized algal biomass has been confirmed to have high capability as a low-cost bioremediation technology for pesticides bioremoval, since these techniques are more sustainable and might encourage the use of microalgae for pesticides bioremediation [34]. The extremely high accumulation capacity of some microalgae for potentially dangerous substances has been also exploited for bioremediation techniques for water [35,36].

Conclusion

It is concluded that the lyophilized biomass achieved removal percentages reached up to 99% of pesticides which was higher than living *C. vulgaris* biomass at the short-term experiments. On the other hand, long-term experiments proved the ability of growing *Chlorella vulgaris* for the removal of pesticides. The present results indicate the possibility of water bioremediation using microalgae for removal of organic pollutants (pesticides) in water.

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