ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Bioremediation of ammonia in river Nile by *Ralstonia pickettii* and *Chryseobacterium gambrini.*

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ABSTRACT

Egypt faces some of the Nile River water pollution problems during low demand period where decreasing the Nile water level and concentrated some pollutants such as ammonia which affected on water quality. *Ralstonia pickettii* (ST.1) and *Chryseobacterium gambrini* (ST.2) were isolated from river Nile during increasing ammonia concentration and identified morphologically using enrichment technique then molecularly using 16S rDNA gene. The isolates were tested against the environmental conditions (pH, temperature and conductivity) during 25 days to detect the most optimum conditions for isolates growth. The tested isolates were evaluated for ammonia bioremediation in aquatic system. The results showed that the optimum degree pH for the growth of Ammonia removal isolates (ST.1) and (ST.2) were 7. The temperature 35°C appeared to be the optimum degree for the growth of isolate (ST.1) while 30°C optimum degree for the growth of isolates (ST.2). *Ralstonia pickettii* (ST.1) and *Chryseobacterium gambrini* (ST.2) have the ability to grow in conductivity 2000µs/cm after incubation period 25 days. Application confirmed that ability of isolates (ST.1) and (ST.2) to remove ammonia concentration were (82%) and (84.5%), respectively while mixture from two isolates was able to remove 91.8% of ammonia concentration. The results showed that isolates have substrate specificity on other substances as chlorinated pesticides and crystal violet dye. In conclusion, bioremediation by mixture of *R. pickettii* (ST.1) and *C. gambrini* (ST.2) isolates were considered to be effective method for ammonia removal in aqueous media (river Nile); this study useful for eradication the ammonia problems in river Nile.

Keywords: Ammonia - River Nile – Bioremediation - Chlorinated pesticides - Crystal violet dye *Ralstonia pickettii - Chryseobacterium gambrini.*

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ISSN: 0975-8585

INTRODUCTION

The Nile River is the most important freshwater resource for life and the main source for drinking and irrigation along the basin from its origin until its estuary in Northern Egypt. The pollution status of the water of the Nile River is an important indicator of water quality [1]. The Nile currently receives enormous amounts of agricultural wastewater that carry various chemical pollutants related to the widespread use of fertilizers and pesticides. Besides great quantities of industrial, municipal, and domestic wastes, storm water runoff is drained directly or indirectly into the Nile [2]. The big challenge is to maintain surface water free of contaminants carried by Drains which cause pollution dilemma in Nile River to protect the environment and human health, it is important to develop methodologies to prevent contaminates from point sources. Bioremediation of chemo-pollutants becomes the method of choice because it is economically feasible and safer than chemical remediation technologies [3]. Studies confirmed that ammonia concentration increased in Rosetta branch during low demand period as discussed by Gad *et al.* 2016 [4]. Exposure to larger amounts of liquid ammonia or ammonium ion in the eyes causes severe eye burns and can lead to blindness and have other effects on the skin, respiratory tract, mouth, and digestive tract [5]. Drinking water, according to health rules, should not contain ammonia of organic origin. In case of ammonia of inorganic form maximum acceptable concentration in drinking water is equal to 0.5 mg/dm^3 according to WHO Guidelines for drinking water and Egyptian standards [6]. Hence, it is important to find a novel technique that is effective in the remediation of Ammonia in environment. Ammonium is a common pollutant that is normally eliminated from wastewater by two processes, nitrification and denitrification [7]. Microbes like chemolitho-autotrophic bacteria converts ammonia into nitrite and nitrates. These nitrates are again converts to nitrogen gas by denitrification process. More over autotrophic nitrifiers cannot tolerate higher concentrations of ammonium and organic loads [8]. The success of bioremediation depends not only on the high degradation ability but also on the stability of active microorganisms under varied conditions, such as changes in pH, Conductivity, temperature and incubation Period; so it was necessary to investigate the effects of various environmental factors on the growth ability of the tested microorganisms [9, 10]. Therefore, this study attempted to isolate and identify bacterial microbes for bioremediation of ammonia in aquatic system, to evaluate ammonia degradation potential of these microbial isolate, also to investigate the optimal environmental factors such as pH, Conductivity, temperature and incubation period on the growth of the tested microbial isolates and finally confirmed that isolates can remove ammonia in presences of chlorinated pesticides and crystal violet dye as substrate specificity.

MATERIAL AND METHODS

Sampling:

Samples were collected according to APHA, 2005 [11] from Inlet of Fewa drinking water treatment plant, Rosetta Branch River Nile (table 1, figure 1)

Table (1): Location of studied plants by GPS.

Plant (GPS)	X	y	symbol
Fewa	268207	3454458	Site 1

Figure (1): Showing sampling site (Inlet of Fewa plant)

Isolation by enrichment culture:

The microbial population containing Ammonium degrading organisms from Rosetta branch river Nile was cultivated on Plat count agar media at 35±0.5°C for 48 h. after that ten isolates were transferred considering morphological characteristics to AOB Selective medium Then incubated at 35±0.5°C for 20 days after that observed the positive bottle by change color to pink after incubation period.

Positive strains were isolated by picking the colonies using sterile needles. The isolates were further purified on complex media using the standard spatial streaking method on solid agar medium plates for the bacterial isolates. Repeated four times to obtained purified bacterial strain. then The isolated colonies were tested for their ability to remove ammonia in AOB broth medium replacing the nitrogen source with different concentration of ammonia (5 mg/l, 10 mg/l, and 20 mg/l) on three glass bottles of volume 200 ml and others three bottles as control without inoculums then incubated at 35±0.5°C for The cultures were shaken at 150 r/min and 35°C for 20 days after that measured the remaining ammonia in three different concentrated bottles by spectrophotometer as described by APHA, 2005 [11].

Identification:

The cultural, morphological and physiological properties of the most efficient ammonia removal isolates were studied to identify these organisms and Comparing the data given with those reported by the investigators according to Bergeys manual of systematic bacteriology 1984 [12] then confirmed genetically using 16S rRNA by Boye *et al*., 1999 [13]. The molecular techniques were performed by sigma, Cairo, Egypt.

Analytical procedure:

The incubated samples were analyzed directly after extracted by dichloromethane and exchange solvent by N. hexane in concentrator (CAPILER turbo 500-USA) put 2ml GC vial extracted sample in auto sampler (CP8300-USA) transferring automatically to column (type CBCIL19) to separate the analyst individually, then Detector electron cubature (ECD) measure the concentrate of its analyst, results appear as pick curves on GC instrument(VARIAN CP 3800-USA). Standard of tested pesticide was prepared from technical grade material and were used wherever cultured samples were analyzed. Ammonia was measured by spectrophotometer on wave length 425 nm while crystal violet was measured on wave length 590 nm as described by APHA, 2005 [11].

Effect of pH, temperature, conductivity and incubation period on the growth of the tested isolates:

To determine the effect of temperature, pH, conductivity and incubation period on the growth of tested isolates, a volume of 200 ml AOB broth media replacing the nitrogen source with ammonia concentration 5 mg/l inoculating with bacterial cell suspension at 10^7 cfu/ml and other bottle 200 ml as control un-inoculate. To determine the optimum pH, experiments were carried out at pH 5.5, 6.5, 7.0, 7.5 and 8.0 and 8. Cultures were incubated on a rotary shaker at 35°C and 150 r/min up to 20 days. To determine the effect of temperature, AOB broth media with pH of 7 was incubated at 20, 23, 26, 30 and 35°C under 150 r/min. to 20 days. To determine the effect of conductivity, AOB broth media with pH of 7 and conductivity was 400, 800, 1000, 1400 and 2000µs/cm incubated at 35°C under 150 r/min for 20 days. To determine the effect of incubation period, AOB broth media with pH of 7 and conductivity was 2000µs/cm incubated at 35°C under 150 r/min for 5,10,15,20 and 25 days.

Bioremediation of ammonia in aquatic system by mixture of *R. pickettii* (ST.1) and *C. gambrini* (ST.1):

Mixture from bacterial cell suspension ST.1 (107cfu/ml) and ST.2 (107cfu/ml) were then used to inoculate 200 ml Raw water from Rosetta branch (site1) as showed in table (1), fig. (1) .ammonia concentration was 17.3 mg/l, the sample site was incubated at 35 °C, and 150 rpm for 25 days and other bottle was control (200 ml un-inoculate).The test principle is based on the decrease of Ammonia concentration was determined by spectrophotometer after incubation period. Remaining value of ammonia measured according to APHA, 2005 [11].

ISSN: 0975-8585

Bioremediation of ammonia in presence of chlorinated pesticide by mixture of *R. pickettii* (ST.1) and *C. gambrini* (ST.1):

Mixture from bacterial cell suspension ST.1 (10^7cfu/ml) and ST.2 (10^7cfu/ml) were then used to inoculate 200 ml AOB Selective media replacing the nitrogen source with ammonia concentration 5 mg/l, replacing the carbon source by 5 Ppm from chlorinated pesticide Mixture, The culture was incubated at 35 °C, pH 7, conductivity was 2000µs/cm and 150 rpm for 25 days and other bottle was control (200 ml un-inoculate). The test principle is based on the decrease of Ammonia concentration was determined by spectrophotometer after incubation period, remaining value of Chlorinated pesticide was determined by Gas Chromatograph. Remaining value of ammonia and Chlorinated pesticide measured according to APHA, 2005 [11].

Bioremediation of ammonia in presence of crystal violet by mixture of *R. pickettii* (ST.1) and *C. gambrini* (ST.1):

Mixture from bacterial cell suspension ST.1 (10^7cfu/ml) and ST.2 (10^7cfu/ml) were then used to inoculate 200 ml AOB Selective media replacing the nitrogen source with ammonia concentration 5 mg/l, replacing the Carbon source by 50 µl from Crystal violet, The culture was incubated at 35 °C, pH 7, Conductivity was 2000µs/cm and 150 rpm for 25 days and other bottle was control (200 ml un-inoculate). The test principle is based on the decrease of Ammonia concentration was determined by spectrophotometer after incubation period, remaining value of Crystal violet was determined by spectrophotometer .Remaining value of ammonia and Crystal violet measured according to APHA, 2005 [11].

Antagonism Test:

To illustrate the model for antagonism between microorganisms, three representative species were tested. These strains are *Ralstonia pickettii* and *Chryseobacterium gambrini*. Nutrient agar media were liquefied and cooled to about 50 °C and inoculated with the initial test organism. These were then poured into sterile Petri plates and allowed to cool at room temperature before streaking with the second test organism. Observations were made after the plates were incubated at 37°C for 48 hours [14].

Statistical analysis:

Data were calculated as mean ± standard deviation (SD). Probability of 0.05 or less was considered significant. The statistical package of Costat Program (1986) was used for all chemo-metric calculations.

RESULTS AND DISCUSSION

Isolation of the ammonia remediation isolates:

From the microbial sources (Rosetta branch site 1) a total of 10 morphologically different Ammonia degradation isolates were obtained. Among 10 bacterial isolates, two bacterial isolates designated as ST.1and ST.2 achieved higher Remediation for Ammonia comparing with the other isolates. The results were compared with those obtained with no inoculated medium (controls).

Identification of the efficient of ammonia remediation isolate:

These bacterial isolates (ST.1) and ST.2 were identified according to morphological, physiological as well as using analysis of 16S rDNA [13]. These efficient Ammonia remediation isolate (ST.1) was white color, gram-negative, motile, rods and Oxidase positive, Non Endospore Catalase positive, Urease positive and hasn't indol production while isolate (ST.2) was purple color, gram-negative, motile, rods and Oxidase positive, Non Endospore Catalase positive, Urease positive and hasn't indol production According to the 16S rDNA analysis, the phylogenetic tree of the Ammonia remediation bacterium isolate ST. 1 and related bacterial species based on the 16S rDNA sequence is provided in Fig (2). It can be clearly seen that the *Ralstonia sp* ST.1. As Ammonia remediation bacterium was included in the genus *Ralstonia* and closely related to the species *pickettii*. It showed the highest sequence similarities with *Ralstonia pickettii* strain JCM 5969 (99%) Figure (2).while the second isolate ST.2 can be clearly seen that the *Chryseobacterium sp* ST.2. As Ammonia remediation bacterium

ISSN: 0975-8585

was included in the genus *Chryseobacterium* and closely related to the species *gambrini*. It showed the highest sequence similarities with *Chryseobacterium gambrini* strain 5-1ST1a (99%) (Figure 3).

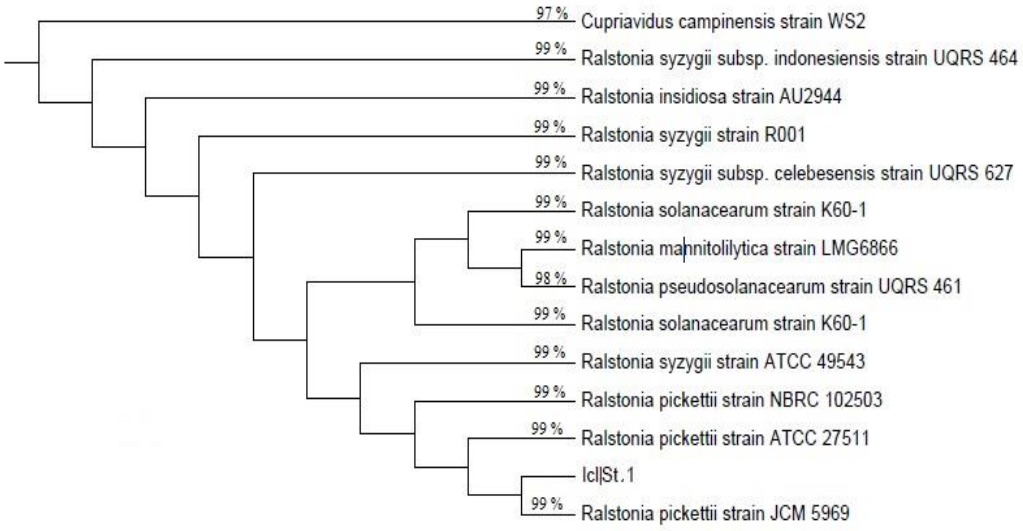

Figure (2): Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA sequences, showing the position of strain *Ralstonia pickettii* (ST.1) among phylogenetic neighbors.

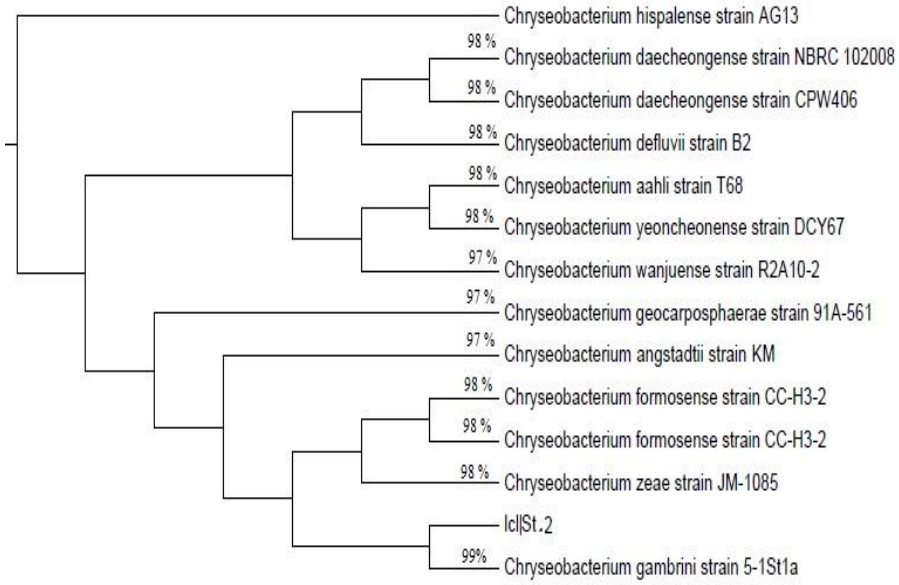

Figure (3): Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA sequences, showing the position of strain *Chryseobacterium gambrini* (ST.2) among phylogenetic neighbors.

Influence of pH, temperature, conductivity and incubation period on the growth of tested isolates:

The influence of pH on ammonia remediation in AOB with *R. pickettii* strain ST.1 and *C. gambrini* ST.2 by using remaining value detection is shown in table (2).in case of ST.1 The highest ammonia remediation with strain was achieved at pH 7; such results were given by Nair *et al.* (2016) [15], while other case ST.2 the highest ammonia remediation with strain was achieved at pH 7 and 7.5; such results were given by Kook *et al.* (2014) [16].

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Table (2): Effect of different pH value on ammonia removal by *R. pickettii* (ST.1) and *C. gambrini* (ST.2)

pH / Strain	Remaining Ammonia (mg/l) at different pH value				
	5.5	6.5	7	7.5	8
R. pickettii (ST.1)	58±0.5	37.6±0.49	26±0.8	32±0.3	35±0.84
C. gambrini (S.T2)	39±0.77	27.4±0.58	22±1.2	21±0.93	30±0.74
Control (un-inoculated)	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0

The effectiveness of biological ammonia oxidation treatment to reduce source water ammonia levels is dependent on temperature [17].The influence of temperature on ammonia remediation in AOB with *R. pickettii* strain ST.1 by using remaining value detection was shown in Table (3). The optimum temperature degree for Bioremediation was 35 ºC these result confirmed by De Baere *et al.* (2001) [18] while in case of *C. gambrini* (S.T2) The optimum temperature degree for Bioremediation was 30 ºC these result agreed by Kook *et al.* (2014) [16].

Table (3): Effect of different temperature degree on ammonia removal by *R. pickettii* (ST.1) and *C. gambrini* (ST.2)

Temperature / Strain	Remaining ammonia (mg/l) at different temperature				
	20°C	23°C	26°C	30°C	35°C
R. pickettii (ST.1)	48±0.4	34±0.8	24±0.3	22±0.6	20±0.55
C. gambrini (S.T2)	32±0.28	24±0.9	18.8±0.95	16.8±1.0	22.8±0.82
Control (un-inoculated)	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0

Effect of Conductivity degrees:

Electrical conductivity (EC) estimates the amount of total dissolved salts (TDS), or the total amount of dissolved ions in the water which affect on permeability of cell membrane of bacteria and changes of ions carrying cause problems in cell membrane of bacteria and their growth [19]. *Ralstonia pickettii* was able to grow in saline solution; such results were given by Labarca, *et al.* (1999) [20]. While the second isolate *C. gambrini* (ST.2) 2000 µs/cm was the optimum conductivity for ammonia bioremediation cm this agreed with Hahnke *et al.* (2015) [21], who confirmed that *Chryseobacterium gambrini* have been isolated from soil, freshwater, marine and saline environments; as shown in table (4).

Table (4): Effect of different conductivity degrees on ammonia removal by *R. pickettii* (ST.1) and *C. gambrini* (ST.2).

Conductivity / Strains	Remaining Ammonia (mg/l) at different Conductivity µs/cm				
	400 µs/cm	800 µs/cm	1000 µs/cm	1400 µs/cm	2000µs/cm
R. Pickettii (ST.1)	28±0.4	24.2±0.6	20.4±0.23	19.9±0.5	19± 0.8
C. gambrini (S.T2)	22±0.12	20±0.54	19.2±0.66	18.5±0.9	17.6± 0.7
Control (un-inoculated)	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0

Effect of incubation period:

In case of first isolate ST.1 the optimum incubation period was 25 days for ammonia remediation such results were given by Becher *et al.* (2000) [22], who confirmed that *Ralstonia sp.* give results in aromatic bioremediation after 48 hr. while remediation improved when incubation period was more than 10 days to 30 days while the second isolate ST.2 the optimum incubation period was 20 days for ammonia remediation by ST.2 isolate this agreed with Ebrahimi *et al.* (2012) [23], who confirmed that *Chryseobacterium sp* growth and hydrocarbon degradation were fluctuated by increasing of incubation time and Colony diameter increased by increasing time of incubation. Largest mean of colony diameter was observed after three weeks of incubation; as shown in table (5).

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Table (5): Effect of different incubation period on ammonia removal by *R. pickettii* (ST.1) and *C. gambrini* (ST.2)

incubation period / Strains	Remaining Ammonia (mg/l) at different incubation period				
	5 days	10 days	15 days	20 days	25 days
R. Pickettii (ST.1)	26.9±0.02	23.7±0.2	20.8±0.05	18.5±0.3	18± 0.1
C. gambrini (S.T2)	20.1±0.15	19.5±0.01	18.3±0.2	16.2±0.14	15.5± 0.2
Control (un-inoculated)	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0

Antagonistic characters between *R. pickettii* (ST.1) and *C. gambrini* (ST.2):

The result showed that, there was no antagonistic reaction between isolate *R. pickettii* (ST.1) and isolate *C. gambrini* (ST.2). Microbes may compete with another microbe for nutrients and these competitions often result to inhibition of one of the microbes. This is termed as antagonism which is described as the inhibition of a bacterium by the products of another; Bacterial antagonism depends on many factors. Among others, for example is a competition for an essential nutrient, the production of toxic metabolites that may inhibit the growth of competing species [24].

Bioremediation of ammonia in aquatic system by mixture of *R. pickettii* (ST.1) and *C. gambrini* (ST.2):

The important process of chemolithotrophic nitrification is a two-step process involving bacterial and archaeal ammonia- oxidizers, oxidizing NH3 to NO2 , and nitrite-oxidizing bacteria, oxidizing NO2 to NO3 [25]. Ammonia oxidation is the first and often the rate-limiting step of nitrification and, therefore, is critical for the global nitrogen cycle [26]. The mixture of two bacterial isolates showed more pollutants degradation than individual strain [27], Mixture of isolates (*R. pickettii* ST.1 and *C. gambrini* ST. 2) have the ability to remove 91.8 % from ammonia concentration in aquatic system after 25 days as showed in table (6). theses result agreed with Clavo *et al.* 2004 [28], who pointed that *Ralstonia Pickettii* sequence showed similarity percentages higher than 91% with bacteria belonging to the β-subgroup of AOB which have ability to nitrify ammonia; when used the sequence of the FISH probeNso1225R as a reverse primer in order to analyze the AOB composition of several environmental samples by denaturing gradient gel electrophoresis (DGGE).While Kundu *et al.* (2014) [29] confirmed that *Chryseobacterium gambrini* successfully stabilized COD as well as ammonium nitrogen. It was capable of utilizing NO3−-N aerobically in presence of NH4 +-N. Nitrogen removal was dependent on the nature of the carbon substrate but independent of the type of the nitrogen substrate. This bacterium is a new member in the group of microbes capable of simultaneous removal of organic carbon and nitrogen from wastewater, the kinetic coefficients of substrate removal and growth for concomitant carbon oxidation, nitrification, and denitrification.

Table (6): The removal percentage of ammonia by mixture of *R. pickettii* (ST.1) and *C. gambrini* (ST.2) in aquatic system during 25 days.

incubation period / Strains	Remaining Ammonia (mg/l) at different incubation period				
	5 days	10 days	15 days	20 days	25 days
Mixture (ST.1)&(ST.2)	59.4±0.18	34.6±0.5	27.8±0.87	14±0.5	8.2± 0.9
Control (un-inoculated)	100±0.5	99.5±0.6	99.0±0.2	98.4±0.14	98.2±0.5
Removal %	49.2 %	65.4 %	72.2 %	86 %	91.8 %

Bioremediation of ammonia in presence of chlorinated pesticide by mixture of *R. pickettii* (ST.1) and *C. gambrini* (ST.2):

Organochlorine pesticides (OCPs; aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, toxaphene, hexachlorocyclohexane) are one of the priority groups of chemical pollutants found in all environmental media [30]. Removal values of Chlorinated Pesticides by *Ralstonia Pickettii* (ST.1) were good with (DELTA Lindan (β -BHC), (DELTA Lindan (δ -BHC), HEPTACHLOR EPOXIDE, ENDOSULFAN-1, ENDRIN ALDEHDE and GAMA Lindan (γ-BHC)); removal values were accepted in case of (DIELDRIN, HEPTACHLOR, DDT, ENDOSULFAN-2 and

ENDOSULFAN SULFATE), while removal values were poor in case of the others Chlorinated Pesticides. the results as shown in table (7) this result confirmed by Ryan *et al.* 2007 [31], who concluded that *Ralstonia pickettii* has the ability to survive and prosper in oligotrophic environment and use a variety of compounds as energy and carbon sources. Such results also were given by Fava *et al.*(1995) [32], who pointed to *Ralstonia pickettii* can utilize chlorinated Compounds as carbon sources and catabolize these compounds without use of supplemental nutrients or cofactors; while Qu *et al.* (2015) [33] confirmed that *Chryseobacterium gambrini* capable of degrading various organochlorine pesticides (OCPs) , 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane (DDD), 1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene (DDE), 1-chloro-2,2-bis (4-chlorophenyl) ethylene (DDMU), 1-chloro-2,2-bis (4-chlorophenyl) ethane (DDMS), and dichlorobenzophenone (DBP) and utilizing them as a sole carbon and energy source for growth.

Table (7): Bioremediation of ammonia in presence of chlorinated Pesticides by mixture of *R. pickettii* (ST.1) and *C. gambrini* (ST.2)

Pesticides types	Chlorinated Pesticides Remaining ppb		Ammonia Remaining mg/l
	Control un-inoculated	mixture (ST.1)&(ST.2)	mixture (ST.1)&(ST.2)
	Remaining %25 day	Remaining %25 day	Remaining %25 day
ALPHA Lindan (α-BHC)	100±0.82	80.8±0.1	9.3±0.49
GAMA Lindan (γ-BHC)	100±0.82	47.4±0.15	9.3±0.49
HEPTACHLOR	100±0.82	55±0.01	9.3±0.49
ALDRIN	100±0.82	77±0.2	9.3±0.49
BETA Lindan (β -BHC)	100±0.82	38.2±0.05	9.3±0.49
DELTA Lindan (δ -BHC)	100±0.82	30±0.3	9.3±0.49
HEPTACHLOR EPOXIDE	100±0.82	36±0.1	9.3±0.49
ENDOSULFAN-1	100±0.82	40±0.2	9.3±0.49
DDE	100±0.82	99.2±0.4	9.3±0.49
DIELDRIN	100±0.82	51±0.13	9.3±0.49
ENDRIN	100±0.82	82±0.01	9.3±0.49
DDD	100±0.82	90.7±0.2	9.3±0.49
ENDOSULFAN-2	100±0.82	52±0.2	9.3±0.49
DDT	100±0.82	53±0.15	9.3±0.49
ENDRIN ALDEHDE	100±0.82	47±0.1	9.3±0.49
ENDOSULFAN SULFATE	100±0.82	54.2±0.11	9.3±0.49
METHOXYCHLOR	100±0.82	80±0.05	9.3±0.49

Bioremediation of ammonia in presence of crystal violet (CV) by mixture of *R. pickettii* (ST.1) **and** *C. gambrini* (ST.2)

The decoloration of organic dyes in industrial waste effluent is essential to a pollution-free environment. organic and synthetic dyes contain numerous toxic substances that cause carcinogenic threats to the health of humans and other mammals [34]. The removal value of crystal violet by Mixture of *R. Pickettii* (ST.1) and *C. gambrini* (ST.2) was good. Such result agreed with Chen *et al.* (2014) [35], who concluded that *R. pickettii* is capable of degrading 2,4,6-trichlo-rophenol , chlorobenzene EV removal, and confirmed that *Ralstonia pickettii* performed demethylation, deethylation, aro-matic ring opening, while Kundu *et al.* (2014) [29] pointed that *Chryseobacterium gambrini* capable of simultaneous removal of organic carbon and nitrogen from wastewater as showed in table (8).

Table (8): Bioremediation of ammonia in presence of crystal violet by mixture of *R. pickettii* (ST.1) and *C. gambrini* (ST.2)

Bacterial strains	Control (un-inoculated)		Mixture of (ST.1) & (ST.2)	
Incubation Days	0 day	25 days	0 day	25 days
CV remaining (mg/l)	100±0.0	99.13±2.0	100±0.0	16.13±0.7
Ammonia Remaining (%)	100±0.0	98.8±2.0	100±0.0	10.1±0.5

ISSN: 0975-8585

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