Poll Res. 40 (3) : 801-808 (2021)

ISSN 0257–8050

REDUCTION OF PERCHLORATE BY FUNGAL SPECIES ISOLATED FROM WATER SAMPLES COLLECTED FROM SOUTHERN KERALA

ANN M. GEORGE[1], K. B. RADHAKRISHNAN[2] AND A. JAYAKUMARAN NAIR[3]

[1]*Lal Bahadhur Sasthri Centre for Science and Technology, University of Kerala, Thiruvananthapuram 695 581, Kerala, India*
[2]*Department of Biotechnology and Biochemical Engineering, SCT College of Engineering, Thiruvananthapuram, 695 018, Kerala, India*
[3] *Department of Biotechnology, Kariavattom Campus, Thiruvananthapuram, 695 581, Kerala, India*

(Received 12 January, 2021; Accepted 25 January, 2021)

ABSTRACT

Perchlorate (ClO_4^-) is a soluble anion that is widely used in explosive industries, rocket fuel manufacturing plants, demilitarization of weaponry and so on. It has been released into the environment for over half a century primarily due to the use of ammonium perchlorate as the propellant in missiles and rockets. The presence of perchlorate can be found in surface and ground water samples. The consumption of perchlorate contaminated water will cause serious health implications in humans by impairing the function of the thyroid gland. Improper thyroid function may affect metabolic functions, growth, cardiovascular and central nervous systems. Perchlorate can also affect the growth and reproductive cycles of aquatic animals also. We found perchlorate concentration from the water samples collected from areas near rocket fuel manufacturing and fireworks above the reference dasage. In the present study, we are dealing with the capability of perchlorate degradation by fungal species *Rhizopus stolonifera* isolated from the contaminated water. The study of the influence of various environmental parameters like concentration of acetate, nitrate, pH, and salinity in the reduction of perchlorate by the fungus. This naturally occurring fungus shows the ability for reducing the perchlorate level within 1 week.

KEY WORDS : Perchlorate, *Rhizopus stolonifera,* Kattaikonam quarry, Puttingaltemple, Acetate

INTRODUCTION

Perchlorate (ClO_4^-) is highly soluble and can react quickly at high temperatures. It is commonly used as an oxidizer in solid propellants, munitions, fireworks, airbag initiatorsfor vehicles, matches and found in some disinfectants and herbicides (Kumarathilaka *et al.*, 2016). Perchlorate can migrate quickly from soil to groundwater from highly contaminated sites (Xu *et al*., 2003). The exposure of perchlorate may inhibit the production of thyroid hormones and higher doses may cause eye and skin irritation, coughing, nausea, vomiting and diarrhea. Furthermore, because of the higher water solubility of perchlorate in water, sufficient rainfall may transport this contaminant away from the source area. According to reported studies, perchlorate has been found in a variety of human body fluids, including urine, breast milk, saliva and blood (Smith *et al*., 2004). Perchlorate can be biologically degraded under suitable condition (Long *et al.,* 2012). The current health advisory level for perchlorate is set at 15 μg/L, based on the reference dose recommended by the United States Environmental Protection Agency (Zewdie *et al*., 2010). Perchlorate contamination due to fireworks production and display is reported from China and Japan and U S A (Isobe *et al.,* 2012). High levels of perchlorate are also detected in ground water samples from a cracker manufacturing place in Tamil Nadu (Anupama *et al*., 2015)

The Ammonium Perchlorate Experimental Plant

(APEP), situated on the banks of river Periyar in Aluva, has been dumping the toxic effluent into the Kochi backwaters. The famous Sivarthri festival is celebrated on the sand banks of Periyar, with fireworks. So is the case with Thrissurpooram. In general, it can be said that fireworks are a part of all most all temple festivals (Utsavams and Poorams) as well as some church festivals. There are hundreds of quarries operating in several parts of the State. The quarries, mostly located in high ranges and foothills of Western Ghats, are a continual source of perchlorate pollution. They also use large quantities of toxic explosives like chlorate and perchlorate for rock cracking. The soil, water and air in these places remain contaminated with perchlorate. Through nonpoint source pollution route, it can reach the fresh water sources and may even contaminate the groundwater, by way of infiltration. The present study has been undertaken to assess perchlorate contamination in surface and ground water from different places in Thiruvananthapuram, Kollam and Pathanamthitta districts and also the ability for the removal of perchlorate by a microorganism found naturally in the contaminated water samples.

Microorganisms can reduce perchlorate, an electron acceptor, to innocuous chloride and oxygen under anaerobic conditions (Bardiya *et al*., 2011). Biological reduction of sodium perchlorate can be done by several species of *Pencillium, Aspergillus* and *Fusarium* (Logan, 1998). The bacterial species capable of chlorate reduction are gram negative, catalase and oxidase positive and motile rods and use oxygen as electronacceptors. A widely accepted perchlorate reducing pathway is given as (Ghosh *et al*., 2011).

$ClO_4^{''}$ (perchlorate) ! $ClO_3^{''}$ (chlorate) ! $ClO_2^{''}$ (chlorite) ! $Cl^{''}$ (chloride) + O_2.

Microbial species such as *Pseudomonas stutzeri* have the capability for degrading perchlorate under aerobic conditions under molybdenum independent environment under room temperature (Shete *et al.*, 2008). In the present study, the fungal species isolated from contaminated water samples undergo perchlorate degradation under aerobic and normal environmental conditions.

MATERIALS AND METHODS

Study area

The water samples collected from southern Kerala, in India 10° 51' 1.8576'' N and 76° 16' 15.8880'' E during January, 2019, Pampa, Kattaikonam, Varkala, Madavurpara, Muthalapozhy and Puttingal. Two or three samples (100 ml) were collected from each point. The samples were taken in pre-autoclaved bottles and transferred to the laboratory in a 24 hr period and filtered. The ground water sources selected are Varkala beach, Madavoorpara which is situated 17 km saway from Trivandrum where the famous temple of Lord Siva is situated and a well in Paravoor near Puttingal Devi temple.

The surface water sources selected were the river Pampa, Kattaikonam quarry and Muthalapozhi coast where we found the dead fish (Krishna *et al*., 2019).

Isolation and screening of perchlorate reducing microorganism

The microorganisms capable of perchlorate reduction was isolated using Inorganic Mineral Media (IMM) (Table 1). Since perchlorate is providing in the media, the organism that can have the ability to reduce perchlorate can be isolated. The plates were incubated under room temperature (37 °C). Microbial colonies isolated from subculture plates were inoculated in inorganic mineral broth and incubated until sufficient growth was obtained. It was then centrifuged and thepelletwas collected for further studies (Anoop *et al*., 2011).

Table 1. Inorganic Mineral Media (IMM) Composition

Ingredients		g/L
1.	K_2HPO_4	- 0.225 g/l
2.	KH_2PO_4	0.225 g/l
3.	$CaCO_3$	0.005 g/l
4.	$FeSO_4.7H_2O$	0.0005 g/l
5.	Yeast Extract	0.200 g/l
6.	$MgSO_4.7H_2O$	0.050 g/l
7.	$KClO_4^-$	1.5 g/l
8.	Sodium acetate	3 g/l
9.	Agar15g/L	

Identification of the isolated organism was done by microscopic observation. The preparation was observed under 40x objective.

Different environmental parametric studies

Environmental parametric studies were conducted in conical flasks tightly stoppered with rubber corks. For all the experiment autoclaved IMM with 100 mg/l initial perchlorate concentration was taken. The studies were conducted under room temperature for an incubation period of 84hrs.To study the effect of acetate on the reduction of perchlorate by the isolated microorganism two different concentrations of acetate were taken in the media 0.1 mg/ml and 0.3 mg/ml. To study the effect of pH on perchlorate degradation was studied by using the pH conditions 4 and 8. The saline tolerant limit of the microbial consortium was studied using the salt NaCl with 1% and 5% w/v. The effect of nitrate was studied by varying nitrate concentration 10 mg/ml and 20 mg/ml.All the above flasks were inoculated with the isolated organism under partial anaerobic conditions and incubated at room temperature. The samples were withdrawn after particular time intervals and checked for perchlorate reduction (Coates *et al.,* 2004).

RESULTS AND DISCUSSION

Identification of Perchlorate in the ground and surface water samples

Preliminary evaluation of the results of this study revealed the presence of detectable levels of perchlorate in all water samples, as may be seen in Fig. 1. It is found that the samples taken from the loading point of Pampa river have a high level of toxic pollution with a pH value of 1.2. The present study indicates highest level of perchlorate contamination in river Pampa had an average value of nearly 820 ±18.5 µg/l where the ammonium perchlorate Plant (APEP) plant at Aluva is located. Perchlorate can form naturally under rare environmental conditions like ozone oxidation of aqueous chloride or through electric discharging of chloride aerosol. During heavy rainfall and floods, the naturally occurring perchlorate will be swept to the water bodies. The discharge of industrial effluents also results in an elevated level of perchlorate concentration in Pampa river water. The ground water sample near APEP plant reported 7270 µg/l and surface water sample reported 355 µg/l of perchlorate concentration. These results are close to the perchlorate concentration near APEP in Ernakulum district (Anupama *et al.,* 2015) and the ground water household well at Thumba, Thiruvananthapuram have a high concentration of perchlorate (300 µg/l) (Anupama *et al*., 2012).

In this study, the water sample collected from a lake close to Kattaikonam quarry shows a very high degree of perchlorate contamination, approximately 670±25.5 µg/l. The direct route of perchlorate contamination from quarries is as follows: Perchlorate, released during explosions in rock quarries, normally gets drained into the quarry lake during rain. Quarrying is found to be a potential source of perchlorate contamination in Palakkad. The quarry water samples near Kattaikonam contained dead fish because of the contamination in the water due to the usage of explosives for the breaking of rocks.

Among the ground water analysed, the well water near Puttingal temple shows the highest level of 150±19.5µg/l of perchlorate contamination and samples from mdavoorpara shows the lowest level of approximately 80±15.4 µg/l Fig. 2. The Puttingal Devi temple in Paravoor where the worst-ever pyrotechnics tragedy that happened in the year 2016. This high level of contamination could be due to the possible infiltration of perchlorate from the nearby blast site. The well which we collected water is situated nearly 200 m away from the site of explosion. Since the location of the well is lower than the site of the explosion in Puttingal Devi

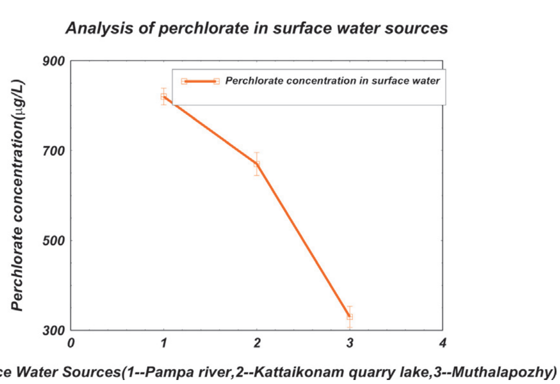

Fig. 1. Perchlorate Concentration level measured from different ground water samples of various sites

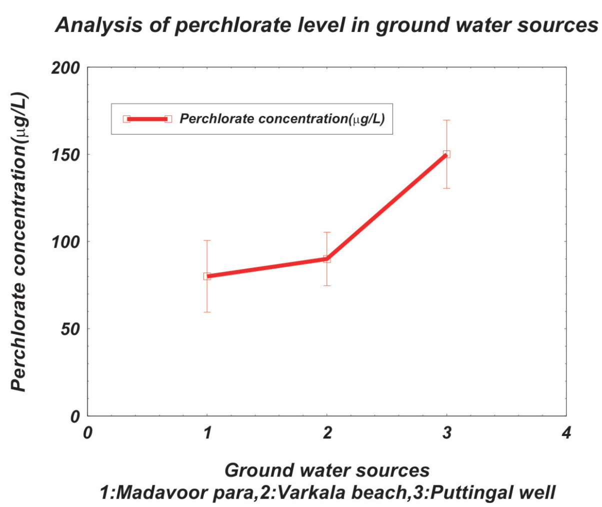

Fig. 2. Perchlorate concentration level of surface water isolated from different sites

temple, there may be chances that the runoff water collected during the rain may directly reach the nearby areas. It is observed in a previous study that water samples from fire crackers in Tamil nadu reported a perchlorate level of 7690 µg/l (Anupama *et al*., 2015).

The samples collected from the Varkala beach also contain 90±20.6 µg/l perchlorate concentration approximately. The Janardana Swami temple is located close to Varkala Papanasambeach. The unavailability of suitable treatment facilities results in sea water pollution (Rajan *et al*., 2013; Kutty *et al*., 2019).

ISOLATION OF PERCHLORATE REDUCING ORGANISM

The perchlorate reducing fungus was identified through Lacto phenol cotton blue method. Here, three type of fungus was identified Fig. 3a, 3b, 3c.

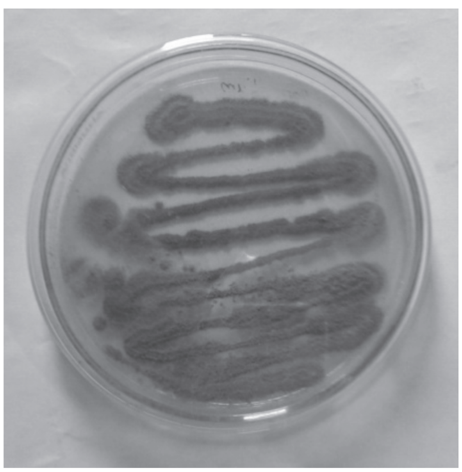

Fig. 3a. *Penicillium*

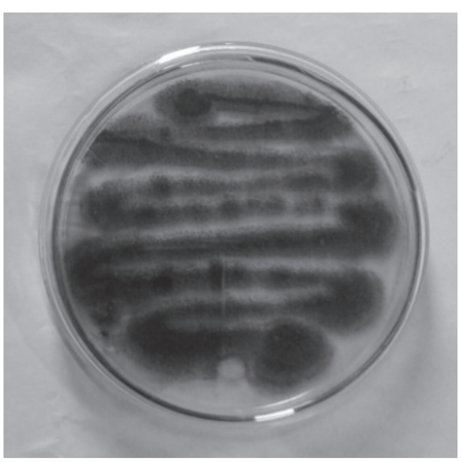

Fig. 3b. *Aspargillus*

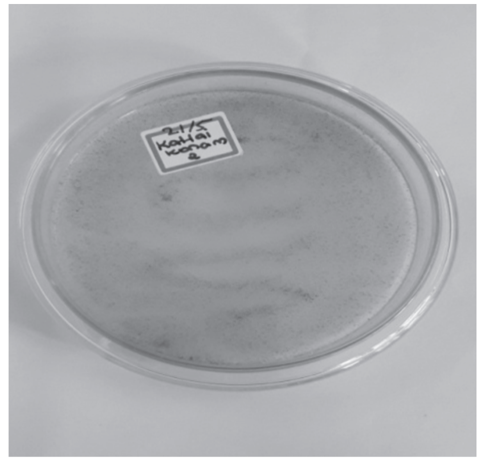

Fig. 3c. *Rhizopus*

Rhizopus- white coloured, *Pencillium*- green colored and *Aspergillus*- black colored. The most effective perchlorate reducing species obtained was from the water sample of the Kattaikonamquarry. The organism shows the ability to degrade the perchlorate level under aerobic conditions. The appearance is such a way that broad hyphae with rhizoids and brown sporangiophores.

After one-week incubation, *Rhizopus* shows 50% perchlorate reduction, *Penicillium* shows 45%, and *Aspergillus* shows 42% perchlorate reduction.

On microscopic observation, the microorganism on the plate was identified as a fungus, *Rhizopus stolonifer* Fig 4. Rhizopus is a type of fungus which belongs to the class *Zygomycetes* because of the fact that it produces zygospore in its sexual reproductive phase. These can reproduce by vegetative, asexual and sexual means followed by fragmentation, sporangiospores and zygospores.

Fig. 4. *Rhizopus stolonifer*

Effect of Environmental Parameters on Perchlorate Degradation

The effect of various parameters such as pH concentration of acetate, nitrate, and salinity on perchlorate degradation has been studied at room temperature (37 ºC) (Anoop *et al*., 2012). Nitrate and acetate act as growth inhibitors. The reduction of perchlorate happens in the absence of oxygen. But the organisms need oxygen for their growth. Thus the oxygen present in the reaction flask will be utilized by the organism for their growth. So after finishing the oxygen in the flasks, the growth of the organism will cease and its ability for reducing perchlorate also reduces. So studies were conducted for a period of 1 week. The samples after 1-week incubation were centrifuged and filtered using asyringe filter and analyzed the reduction level of perchlorate using an ion analyzer. The experiments were conducted at room temperature (37 °C).

Effect of Acetate

Since acetate was found to be the best carbon source for perchlorate reduction, studies were done to find out the percentage reduction of perchlorate with different acetate concentration, shown in Fig. 5. Periodically perchlorate reduction was measured with the help of anion analyzer.

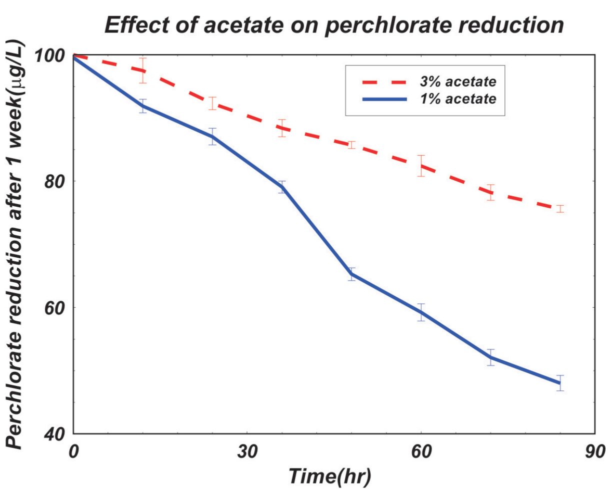

Fig. 5. Effect of acetate on perchlorate degradation

After 1 week of incubation, the cultures were centrifuged and filtered. The perchlorate found in the given filtered samples was measured using anion analyzer. It was observed that as the concentration of acetate in the medium increases, the reduction of perchlorate decreases. This can be due to the inhibition of fungal growth byacetate in the medium. For a typical case the perchlorate reduction rate is 48±1.21 µg/l and 75.6±1.21 µg/l for 1% and 3% acetate concentration, respectively. For 3% acetate, the reduction level is only 40% compared with 1% acetate, it is more than 60% after 1-week incubation. Previous studies have shown that the presence of sodium acetate affected the growth of several strains of *Rhizopus*. Because sodium acetate has an antifungal property (Stles *et al.,* 2002). But Microbial studies also reveal that acetate is essential for perchlorate degradation (Mamie *et al*., 2005).

Effect of nitrate

The burning of the rocket fuel leads to the simultaneous release of nitrate along with perchlorate. Thus nitrate and perchlorate are coexisting. The experiments were conducted to estimate the effect of nitrate on perchlorate degradation by taking nitrate concentrations 5,10 and 20 mg/l. After an incubation period of 1 week the perchlorate reduction was measured.

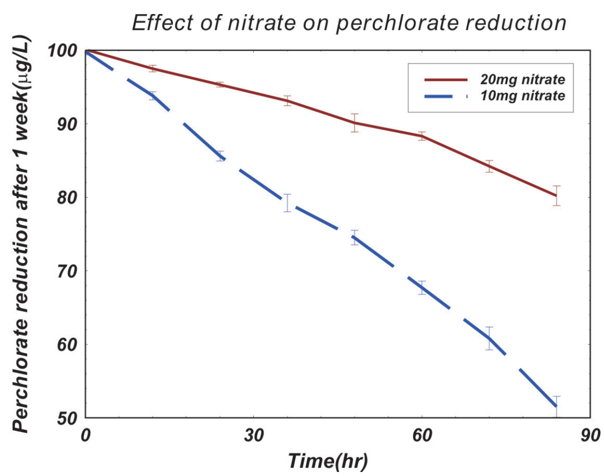

Fig. 6. Effect of nitrate on perchlorate degradation

Normally nitrate promotes perchlorate reduction at a lower level of nitrate and inhibits the reduction level at higher concentrations. High nitrate loadings significantly shape the biofilm microbial community (Cang *et al*., 2004). As per the results shown in Fig. 6 the reduction rate of perchlorate decreases with increase in nitrate concentration. The maximum perchlorate degradation was observed for 10 mg/ 100 mL nitrate and the rate of degradation decreases as the amount of nitrate in the media increases. At an incubation period of 84 hrs for 10 mg/100 mL nitrate concentration, the reduction of perchlorate is

51.5±1.43 μg/l and for 20 mg/100 ml nitrate concentration the reduction of perchlorate is 80.2±1.33 μg/l. This shows that nitrate acts as an inhibitor on the perchlorate degradation mechanism (Li *et al.*, 2019). So for getting maximum degradation using the organism, the presence of nitrate should be minimum.

Effect of pH

Experiments were conducted in acidic and basic pH as shown in Fig. 7. Results show that the maximum perchlorate degradation was found to be at pH 4.0 and there was a decrease in the rate of perchlorate reduction when pH moves to basic side. When the time of incubation is 84 hrs the rate of perchlorate reduction at pH 4 is 50.5±.93 μg/l and 80.6±1.23 μg/l in the case of basic pH. At neutral pH it is 72.4±.79 μg/l. Most fungi prefer acidic pH for their growth and reproduction (Thalisa *et al.*, 2018). Studies conducted by Hassan *et al.*, 2017 on the environmental factors affecting the growth of pathogenic fungi have shown that *Rhizopus stolonifer* has its highest growth at pH 5.5, and the growth declined at neutral pH. In the studies conducted by Amiri *et al.*, 2011, on the effect of pH on growth and germination of *Rhizopus stolonifer* showed that the growth was totally inhibited below pH 2.5 and growth happens between pH 3 and 10. So in the present study, the fungal growth and perchlorate reduction happen most, preferably at acidic pH.

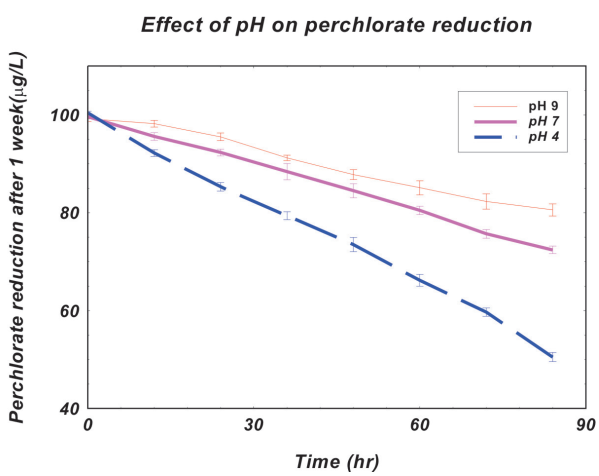

Fig. 7. Effect of pH on perchlorate degradation

Effect of Salinity

To study the effect of varying salinity on ClO_4^- reduction, batch studies were conducted and results are shown in Fig. 8. Results showed that there was a considerable decrease in the rate of ClO_4^- reduction when the salinity increases. The increase in salinity of media may cause biochemical changes in fungi (Venacio *et al.*, 2017). The effect of salinity also influences the production of secondary metabolites of fungal species (Huang *et al.*, 2011). For a typical case of 5% NaCl the average value of perchlorate reduction is 81.9 ±1.22 μg/l and for 1% NaCl it is 53.5 ± 1.55 μg/l. The perchlorate reduction percentage reaches 70% on 1% salinity and declines as the salinity in the medium increases. Studies have shown that high salinity decreases fungal growth and germination of spores (Boumaaza *et al.*, 2015).

CONCLUSION

The present study shows that perchlorate concentration is relatively low in ground water when compared to the surface water. Among the surface water samples, the highest concentration of perchlorate was observed in Pampa River followed by Kattaikonam quarry. The ground water samples had the lowest concentrations of perchlorate. This indicates the perchlorate concentration in water sources decreases with increasing depths. This study also reveals that the level of contamination depends upon the distance from the source of contamination. In this study, the water sample collected from a lake close to Kattaikonam quarry shows avery high degree of perchlorate contamination, approximately 670 μg/l. The present study indicates the highest level of perchlorate contamination in river Pampa had an average value of nearly 820 μg/l.

Present study has attempted to emphasize the isolation of perchlorate degrading microorganism

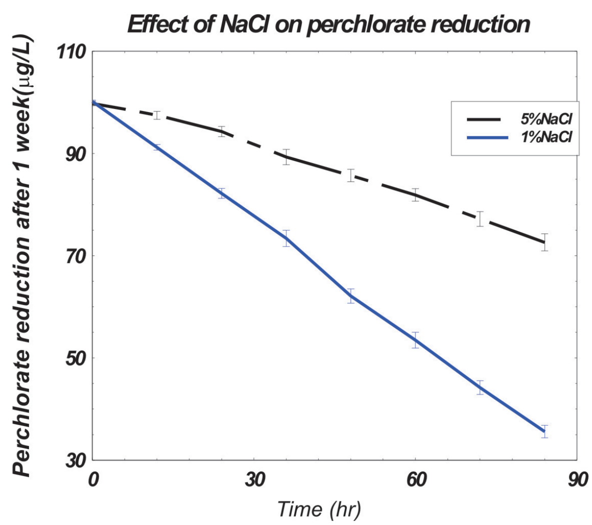

Fig. 8. Effect of salinity on perchlorate degradation

and the various environmental factors affecting biodegradation. Microorganisms were isolated from water samples of perchlorate contaminated sites and found that these fungal species have the ability to reduce perchlorate under natural environmental conditions. Samples from Kattaikonam reduced ClO_4^- more effectively than other fungal species and it was identified as *Rhizopus stolonifer.*

Batch reactor results suggest that the ClO_4^- degradation depends on the influence of acetate concentrations and concentration of alternate electron acceptors such as oxygen, presence of oxygen scavengers like sulfide, pH, salinity etc. Based on data obtained in a series of batch tests, nitrate and acetate act as an inhibitor in the perchlorate degradation mechanism. It is suggested that the inhibitory effect of nitrate in ClO_4^- reduction is due tothe toxic effect of accumulating nitrite (as a protonophore) in the medium rather than competition with NO_3 for electron donor. The result shows that acetate is a very effective carbon source and electron donor for ClO_4^- reduction. From the result, it was clear that there is a decrease in the rate of perchlorate reduction when pH moves to the basic side. Perchlorate reduction under high saline conditions shows that salinity has got a significant effect on perchlorate degradation. As salinity increases, the ClO_4-degradation decreases.

REFERENCES

Amiri, A., Chai, W. and Schnabel, G. 2011. Effect of nutrient status, pH, Temperature and Water potential on germination and growth of *Rhizopus stolonifer* and *Gilbertella persicaria*. *Journal of Plant Pathology.* 93 (3) : 603-612.

Anoop Raj, J.R. and Muruganandam, L. 2011. Biodegradation of perchlorate by methylophaga sp. lmn developed from an acclimatized mixed. *Journal of Chemical, Biological and Physical Sciences (JCBPS).* 2(1) : 359.

Anoop Raj, J.R. and Muruganandam, L. 2012. Batch studies and effect of environmental parameters on microbial degradation of perchlorate using acclimatized effluent sludge. *Journal of Chemical Environment.* 16 (1) : 44-50

Anupama, V.N., Kannan, K., Prajeesh, P.V.G., Rugmini, S. and Krishnakumar, B. 2012. Perchlorate, Chlorate and bromate in water samples from the South wet coast of India. *Water Supply.* 12 (5) : 595-603.

Anupama, V.N., Krishnakumar, B. and Prajeesh, P.V.G. 2015. Surveillance of perchlorate in ground water, surface water and bottled water in Kerala, India. *Journal of Environmental Health Science and Engineering.* 13 (56) : 2-6.

Bardiya, N. and Bae, J.H. 2011. Dissimilatory perchlorate reduction: a review. *Microbiological Research.* 166 (4) : 237-254.

Boumaaza, B., Benkhelifa, M. and Belkhoudja, M. 2015. Effects of two salta compounds on Mycelial growth sporulation and spore germination of six isolates of Botrytis cinerea in the western north of Algeria. *International Journal of Microbiology.* 1-8

Cang,Y., Roberts, D.J. and Clifford, D.A. 2004. Development of cultures capable of reducing perchlorate and nitrate in high salt solutions. *Water Research.* 38 (14-15) : 3322-3330.

Coates, J.D. and Achenbach, L.A. 2004. Microbial perchlorate reduction: rocket-fuelled metabolism. *Nature Reviews Microbiology.* 2 (7) : 569.

Ghosh, A., Pakshirajan, K., Ghosh, P.K. and Sahoo, N.K. 2011. Perchlorate degradation using an indigenous microbial consortium predominantly *Burkholderia* sp. *Journal of Hazardous Materials.* 187 (1-3) : 133-139.

Hassan, N., Shafiq-Ur-Rehman and Bano, H. 2017. Environmental factors affecting growth of Pathogenic fungi causing fruit rot in tomato (*Lycopersion esculentum*). *International Journal of Engineering Research and Technology (IJERT).* 6 (20) : 578-584.

Huang, J., Lu, C., Qian, X. and Huang, Y. 2011. Effect of salinity on the growth biological activity and secondary metabolites of some marine fungi. *Acta Oceanologica Sinica.* 30 (3) : 118-123.

Isobe,T.,Ogawa, S.P., Sugimito, R. and Ramu, K. 2012. Perchlorate contamination of ground water from fire works manufacturing area in South India. *Environmental Monitoing and Assessment.* 185 (7) : 2-12.

Krishna, R. P., Limisha, A.T., Arun, T.J., Aneesh, T.D., Silpa, B.L., Sreeraj, M.K. and Reji, S. 2019. Accumulation of trend of heavy metals in the surficial sediments of muthalapozhi near shore,South west coast of India. *International Journal of Scientific and Engineering Research.* 10 (6) : 1706-1718.

Kumarathilaka P, Oze C., Indraratne, S.P. and Vithanage, M. 2016. Perchlorate as an emerging contaminant in soil, water and food. *Chemosphere.* 150 : 667-677.

Kutty, S.N. and Sebastian, C.D. 2019. Microbial pollution assessment in major tourist beaches of Kerala. *Online International Interdisciplinary Research Journal.* 9 (4) : 29-34.

Li, H., Zhou, L., Lin, H., Zhang, W. and Xia, S. 2019. Nitrate eefects on perchlorate reduction in a H2/ CO_2-basd Biofilm. *Science of the Total Environment.* 694 (2019) : 133564.

Logan, B.E. 1998. A Review of Chlorate-and Perchlorate

-Respiring Microorganisms, *Bioremediation Journal.* 2 (2) : 69-79.

Long, Y., Hong, Y., Jie, Y. and Huailong, S. 2012. Water treatment technologies for perchlorate: a review. *Desalination.* 298 : 1-12.

Mamie Nozawa-Inoue, Kate, M.S. and Dennis, E.R. 2005. Reduction of Perchlorate and Nitrate by Microbial communities in Vadose soil. *Applied and Environmental Microbiology.* 71 (7) : 3928-3934.

Rajan, B.,Vincy, M.V. and Pradeep Kumar, A.P. 2013. Original Research Article Coastal water Receation and public health of South India A case study on Varkalabeach. *International Journal of Current Microbiology and Applied Sciences.* 2 : 247-255.

Shete, A., Mukhopadhyaya, P.N., Acharya, A., Aich, B.A., Joshi, S. and Ghole, V.S. 2008. Aerobic reduction of perchlorate by bacteria isolated in Kerala, South India. *Journal of Applied Genetics.* 49 (4) : 425-431.

Smith, P.N., Yu, L., Mc Murry, S.T. and Anderson, T.A. 2004. Perchlorate in water, soil, vegetation, and rodents collected from the Las Vegas Wash, Nevada, USA. *Environmental Pollution.* 132 (1): 121-127.

Stiles, J., Penkar, S., Plockova, M., Chumchalova, J. and Bullerman, L.B. 2002. Antifungal activity of sodium acetate and *Lactobacillus rhamnosus*. *Journal of Food Protection.* 65 (7) : 1188-1191.

Thalisa, Y.A. and Kannika, C. 2018. Bioconversion of waste cooking oil glycerol rom cabbage extract to lactic acid by Rhizopusmicrospores. *Bazilian Journal of Microbiology.* 49 (1) : 178-184.

Venacio, C., Pereira, R., Freitas, A.C., Rocha-Santost, A.P., Costa, J.P., Duarte, A.C. and Lopes, I. 2017. Salinity induced effects on the growth rates and mycelia composition of basidiomycete and zygomycete fungi. *Environ Pollution.* 231 (2) : 1633-1641.

Xu, J., Song, Y., Min, B., Steinberg, L. and Logan, B.E. 2003. Microbial degradation of perchlorate: principles and applications. *Environmental Engineering Science.* 20 (5) : 405-422.

Zewdie, T., Smith, C.M. and Hutcheson West, C.R. 2010. Basis of the Massachusettes Reference dose and drinking water Standard for perchlorate. *Environmental Health Perspective.* 118 (1) : 42-48.