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Water Soluble Polyaniline as Biocide

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The microbial fouling of a submerged object is a complex phenomenon and its control requires the use of potentially hazardous chemicals. The effective control of microbial films on a submerged object is essential to prevent its corrosion. The microbial fouling not only accelerates the rate of corrosion due to imbalance of oxygen by creating electrochemical cell. The currently available biocides are for the control of planktonic microorganisms is based on tri-organic tin compounds, which are not friendly to aquatic life. Our preliminary study indicates that polyaniline doped with functionalized dopants at concentration of 4×10^{-6} g/ml is able to control the growth of microorganism in the area of 140mm^2. In this paper we present our study on synthesis and use of water-soluble polyaniline as a biocide to control bio fouling.

Keywords : *aqueous polyaniline, antimicrobial, microorganism, aromatic sulphonic acid.*

1. Introduction

Microorganisms are all pervading including every part of living and non-living objects. Their natural habitation is in the soil and water basin of the earth. Their degree of activity depends on their environment as it relates to physico - chemical nature of the environment such as organic matter, availability of oxygen, moisture, acidity, alkalinity, temperature and host of other unknown parameters. Since water is the basis of all life, microbial growths inevitably occur in its presence. Thus water is the keystone of their existence. In the absence of water, microbial life is impossible. The amount of moisture present in the given commodity determines whether it is attacked by bacterial or fungi. The moisture contents of the commodity if is between 15-35% it will support the growth of fungi and if exceeds 35% it will support bacterial growth. This description of growth of microorganism shows that control of its growth is prehistoric. The man started, to control the growth of microorganism via antiseptics, creosote, which contains a mixture of phenols and carbolic acid to be used as a deodorant to prevent foul odours from sewage and wound infections, which were considered a form of putrefaction. S.C. Block has reviewed the history of modern bacteriology-pure cultures, solidified media and sterile technique and the foundation of modern bio-deterioration. This study shows that bacteria are not all killed at a time, but at a rate that depends on the concentration of the disinfectant and on the temperature.[1]

Along with the protection of life the protection of materials from corrosion and bio fouling assumes significance for the advancement of human development. Thus the protection of materials from deterioration caused by the growth of microorganism on submerged structure is not only of strategic importance but has implication on growth of an industry. At present organo-metallic compounds based on tin and antimony are used as an inhibitors to check the growth of microorganism, however, these organo- metallics are toxic to aquatic life and therefore, application of these material is limited.[2,3]

Industrial water treatment is mostly about managing surface fouling processes. There are three such process to manage namely microbial, scaling and corrosion, which occurs simultaneously. Of these three, the microbial fouling process is the complex and difficult to measure in real terms. It requires the use of the potentially hazardous products and is the least understood. Effective key to successful water treatment is via formation of micro biofilm. The scale and corrosion inhibition chemicals do not work unless microbial fouling is properly controlled. In recent times conducting polymers have gained the importance as new smart materials whose properties can be tailored as per need and environmentally friendly and their synthesis does not require elaborate arrangement.[4] In this communication we present our study on use of polyaniline as an anti-microbial new material, which is not harmful to other aquatic lives.

Conducting polymers, which are conjugated systems and can be made conducting via process known as doping. Due to extensive conjugation these materials suffer with one disadvantage that they cannot be processed by normal methods.[5] However, among these conducting polymers polyaniline has a special status due to the presence of -NH - group flanked on either side by benzene ring, thus structure has a chemical flexibility[6] to yield a water-soluble polymer. Previously we have shown that this water solution can be used effectively as acid corrosion inhibitor for mild steel[7,8] In this communication we present our results on the study of water soluble polyaniline as a new material to prevent the growth of microorganism on the submerged substrate keeping in mind new environmental concerns among scientists and masses. Polyaniline has been known almost for the 130 years as an aniline black.[9] However, the systematic synthesis and structure are of recent origin. Polyaniline though is environmentally stable but it is intractable. Polyaniline is built up from reduced (-B-NH-B-NH-) and oxidised (-B-N=Q=N-) repeating units, where B denotes benzenoid and Q denotes quinoid ring. Thus the ratios of amine to imine yields various structure such as leucoemeraldine (fully reduced form), emeraldine base (neutral, undoped form), emeraldine salt (50% oxidised, doped form) and pernigraniline (fully oxidised form) and its synthesis is straightforward. The structure of various forms is shown in Fig. 1.

A number of water-soluble homopolymers, copolymers and polymer blends of aniline have been reported in the literature.[10-18] The three general approaches to these aniline polymers are:

* By introducing hydrophilic substituents in polyaniline backbone.
* Polymerisation of aniline derivatives containing hydrophilic substituents.
* Ionic doping of parent polyaniline by blending with water-soluble polymeric acid.

The first approach was accomplished by Hany and Genies,[10] by heating the emeraldine salt of polyaniline in dimethyl sulfoxide with either 1,3-propane sulfone or 1,4 -butane sulfone to produce the corresponding poly (aniline-N-propylsulfonic acid) or poly (aniline-N- butylsulfonic acid). Sulfonic acid groups can also be substituted onto the phenyl ring of polyaniline by treating emeraldine base with fuming sulfuric acid.[11]

The second appoarch is polymerization of acid functionalized anilines such as aniline-N-butylsulfonic acid, o-anthranilic acid, o-metanilic acid, and aniline-N-phenyl- p-sulfonic acid.[12-15] The aniline derivatives can also be copolymerized with aniline to form copolymers with high molecular weight.[16]

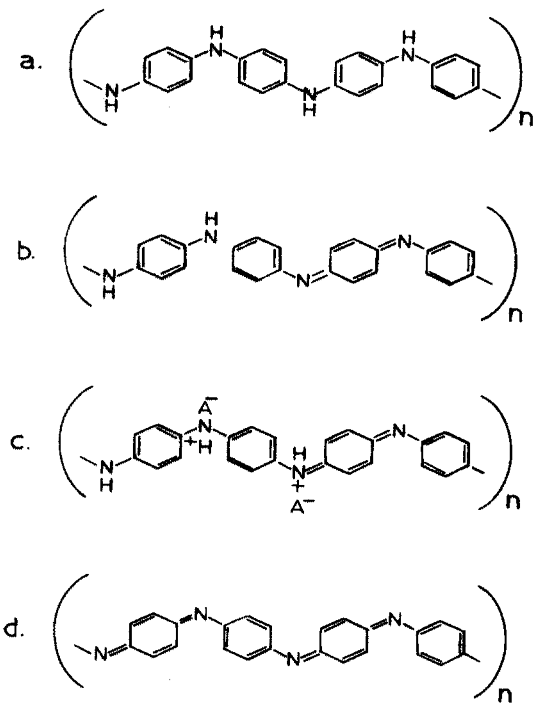

Fig. 1. Various forms of polyaniline. (a) ieuco emeraldine, (b) emeraldine base, (c) conducing emeraldine, and (d) pernigraniline.

The third approach is by chemical polymerisation of aniline and its derivatives in an aqueous solution of a polymeric acid, such as polyacrylic acid or poly (styrene-p-sulfonic acid).[17,18] All these methods yield a polymer having not more than 30 monomer units.

In the present investigation the soluble conducting polyaniline was synthesised by using dopants, which exhibits surfactant property such as alkyl substituted aromatic sulphonic acid as electrolyte cum dopant. Our investigations on this water soluble polyaniline by spectroscopic and chromatographic techniques shows that doped polyaniline has a molecular weight around 1 lakh, which is much higher than till now investigated systems this higher molecular weight is reflected in cyclic voltammetric studies where the peak due to oxidation to macro cation is observed at + 0.8V versus SCE, similarly the absorption spectra shows charge carrier band at 1200 nm. This water-soluble polyaniline has been studied for its anti-microbial properties using eleven types of bacterial species as given in a Table. Out of eleven species six species are gram -negative and the five species are gram -positive, the ten of them are having rod like structures and one species has coccus structure.

2. Experimental

All of the chemicals used were of AnalaR grade. The aniline was freshly distilled under vacuum and kept in nitrogen atmosphere in the dark.

Water soluble polyaniline is prepared by chemical oxidative polymerisation of 0.1M aniline in 1M aqueous aromatic sulphonic acid medium under vigorous stirring for a period of 4 - 6 hrs at a temperature of 0 - 50C by adding drop by drop of 0.1M aqueous solution of ammonium persulphate. The polyaniline was obtained as a clear green solution, which could be freely diluted with water without any precipitation.

Nutrient agar medium was prepared with the composition of peptone (0.5%), yeast extraction (0.15%), beaf extract (0.15%), sodium chloride (0.5%), agar- agar (1.5%) and distilled water. The medium was sterilised in an autoclave at 15lps for 20 minutes then it was cooled at room temperature and then it was plated on a sterile petri dish. A sterile cotton swab was dipped in to the overnight cultures, using the swab, the entire agar surface was streaked in a horizontal direction and then vertically to a heavy growth over the entire agar surface. The plates were then allowed to dry for about 5 minutes and then the polyaniline discs were placed on the agar surface at equal distance by sterile forceps dipped in alcohol. The discs were placed gently with sterile forceps to ensure that the discs adhere to the surface of the agar. The plates were incubated at 37±10C for 24 hrs. Microorganisms were procured from Microbial Type Culture Collection [MTCC] Chandigarh. The following bacterial species were used Cellulomonas cartae 23,Bbacillus sphaericus 1486, Aeromonas hydrophila 1739, Xanthomonas maltophila 434, Bacillus thuringiensis 869, Bacillus megaterium 2412, salmonella typhimurium 98, Agrobacter tumefaciens 2251, streptococcus mutans 890, Klebsiella pneumoniae 109 and Enterofacter aerogens 111 were used for anti microbial assay. The pure bacterial cultures were inoculated in a nutrient broth medium aseptically and it was incubated at 37±10C in a rotary shaker for 18-24 hrs.

The anti-microbial activity of these systems was tested by using Kirby-Bauer method with different types bacterial species found in marine condition. It can be found from the determination of the diameter of the zone of inhibition. This was then compared with the standard. Based on this comparison, the test organism was determined whether resistant or intermediate or sensitive to polyaniline. If it is less than 3mm zone the microorganism are resistant.

3. Results and discussion

Natural strategies to control microbial fouling often involve oxidation in presence of a surfactant. The halogens are widely spread and some algae under aqueous conditions oxidise them to hypohalites, which bound surfactant released by aquatic species to act as natural biocide. Though chlorine is much in abundance it is the bromine, which gets preferentially oxidised due to its low oxidation potential, which can be represented as

$$Br_2\ (l) + 2e^- \rightarrow 2\ Br^- \quad E_0 \text{ is } 1.0652$$

The mechanism of hypohalites to act as effective biocides involves the attack of hypohalite on bacterial membrane thereby membrane bound proteins becomes dysfunctional to distrupt the metabolism.[19]

The oxidation potential of polyaniline in presence of aromatic sulphonic acid is + 0.8V Vs saturated calomel electrode (SCE), which means standard potential E_0, is 1.0444 at 25°C as indicated by cyclic voltammetric studies. This study indicates that our system has a equal potential as given by free bromine to act as biocide with a advantage that polyaniline in addition to its biocidel activity also acts as a surfactant as indicated by our experiments.

The results obtained on anti-bacterial activity of polyaniline on eleven type of bacterial species are recorded as inhibition zone (mm) are tabulated in Table 1. The maximum anti-microbial activity was observed for Cellulomonas cartae 23 using aqueous solution of polyaniline with a concentration of 4×10^{-6} g /ml, the growth of this bacteria inhibits in an area of 30mm. For other species No. 3, 4, 8, 9, 10 and 11 inhibition zone lies between 20 to 25 mm. However, the species No. 5, 6 and 7 have shown only 15 to 16mm of inhibition zone. This calls for further studies on composition of bacterial colonies.

Table 1. Antimicrobial activity of the soluble polyaniline at the concentration of 4x10-6 g/ml

S.NO	Bacterial species	Morphology	Classification	Inhibition zone (mm)
1	Cellulomonas cartae 23	Rod	Gram – negative	30
2	Bacillus sphaericus 1486	Rod	Gram – positive	25
3	Aeromonas hydrophila 1739	Rod	Gram – negative	20
4	Bacillus thuringiensis 869	Rod	Gram – positive	20
5	Xanthomonas maltophilia 434	Rod	Gram – negative	16
6	Bacillus megaterium 2412	Rod	Gram – positive	15
7	Salmonella typhimurium 98	Rod	Gram – negative	16
8	Agrobacter tumefaciens 2251	Rod	Gram – positive	22
9	Streptococcus mutans 890	Coccus	Gram – positive	22
10	Klebsiella pneumoniae 109	Rod	Gram – negative	21
11	Enterofacter aerogens 111	Rod	Gram – negative	20

4. Conclusion

Our study suggests that the polyaniline doped with long chain aliphatic substituted aromatic sulphonic acid acts as biocide due to its excellent reversible redox behaviour coupled with surfactant action under neutral conditions. The more studies are required establish this system as a biocide. At present our experiment are in progress to study the properties of polyaniline coatings on metallic and non-metallic substrates.

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References

1. S. C. Block, in "Historical review" Disinfection, Sterilization and Preservation (Lea & Febgier, 3rd ed.), Philadelphia, 1983.
2. Bergey's Manual of systematic Bacteriology (J. G. Holt, 8th ed), Willams & Willkins Co, Baltimore, 1984.
3. Fundamentals of Microbiology (I. E. Akamo, 4th ed.), the Benjamin / Cummings Publishing Co, 1994.
4. D. C. Trivedi, in Handbook of Conductive Organic Molecules and Polymers Vol. 2(H.S. Nalwa, ed.), John Wiley & Sons, U.K p. 505, (1997).
5. D. C Trivedi, *Bull.Mater. Sci.*, **22**, 447 (1999).
6. D. C. Trivedi, *Ind. J. of Chem.*, **33A**, 552 (1994).
7. S. Sathyanarayanan, S. K. Dhawan, K. Balakrishnan, and D. C. Trivedi, *Corrosion Sci.*, **33**, 1831 (1992).
8. S. Sathyanarayanan, S. K. Dhawan, K. Balakrishnan, and D. C. Trivedi, *Electrochimica. Acta.*, **39**, 831 (1994).
9. H. Letheby, *J. Chem. Soc.*, **15**, 161(1862).
10. P. Hany and E. M. Genies, Synth. Met., **31**, 369 (1989).
11. J. Yue, A.J. Epstein, and A. J. MacDiarmid, *Mol. Cryst. Liq. Cryst.*, **189**, 255(1990).
12. E. Kim, M. H. Lee, B. S. Moon, C. Lee, and S. B. Rhees, *J. Electrochem. Soc.*, **141**, L26(1994).
13. H. S. O. Chan, S. C. Ng, W. S. Sim, K. C. Tan, and B. T. G. Tan, *Macromolecules*, **25**, 6029 (1992).
14. J. Y. Lee, C. Q. Cul, X. H. Su, and M. S. Zshou, *J. Electroanal. Chem.*, **360**, 177 (1993).
15. C. DeArmitt, C. P. Armes, J. Winter, F. A. Uribe, J. Gotterfold, and C. Mombourquette, *polymer.*, **34**, 158 (1993).
16. S. K. Dhawan and D. C. Trivedi, Synth. Met., **60**, 63 (1993).
17. M. Angelopoulos, N. Patel, J. M. Shaw, N. C. Labianca, and S. A. Rishton, *J. Vac. Sci. Technol.*, **311**, 2794 (1993).
18. K. Shannon and J. E. Fernandez, *J. Chem. Soc*, Chem. Commun., 643 (1994).
19. I. R. Wever, *Sci. Technol.*, **25**, 446 (1991).