Application of Antibacterial Ability of Metals for Bacterial Control "A Viable Strategy to Control Bacterial Attachment and Microbiologically Influenced Corrosion"

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OUTLINE

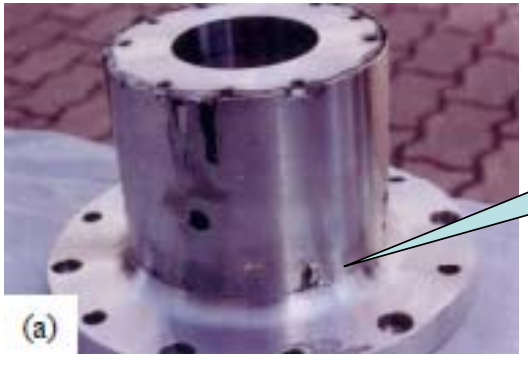
- Background from our research
  The basic MIC research in Stainless Steel welds

- Aim strategy to control bacterial attachments
  - Sulfur
  - Nitrogen

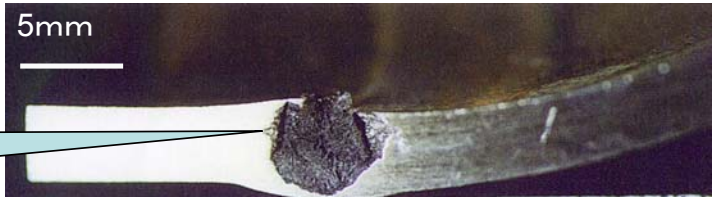
- Experiment to check the ability to reduce bacterial attachments
  - Some pure metals / Japanese industrial standard test methods
  - Commercial product SS / Exposure test in lab. and field
    Copper contained SS
    Silver contained SS

INTRODUCTION

- Microbiologically Influenced Corrosion (MIC) is reported to occur in various types of construct materials.
- Serious MIC failures occur in stainless steel welds.



Type 316L SS
Waste treatment plant

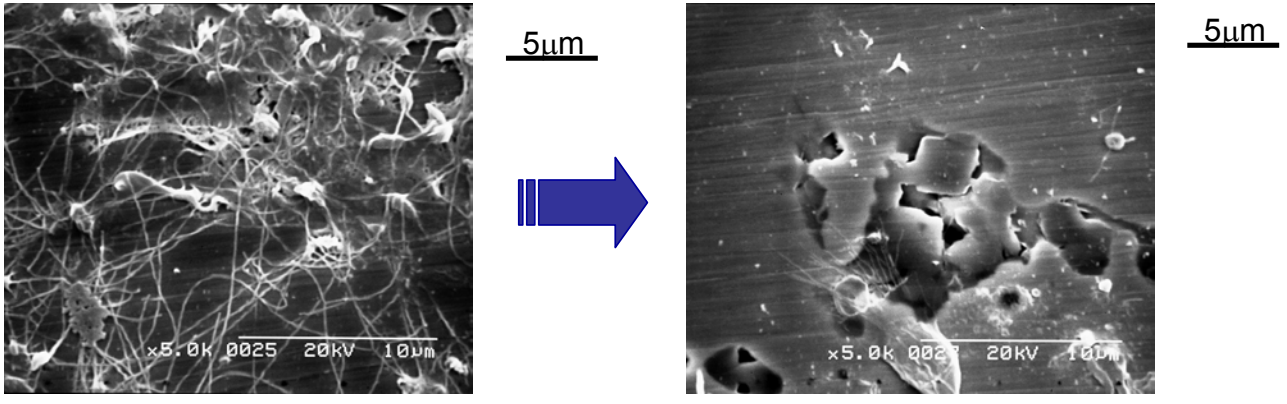


Type 316L SS
Power plant

MIC is one of major concerns in material engineering.

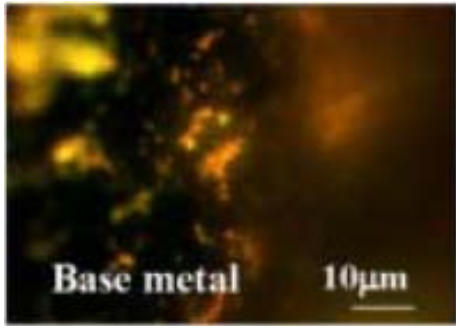
BACKGROUND 1

- When a metal surface comes in contact with a non-sterile fluid, a conditioning film forms, and biofilm formation starts.
- This might result in the initiation of MIC.

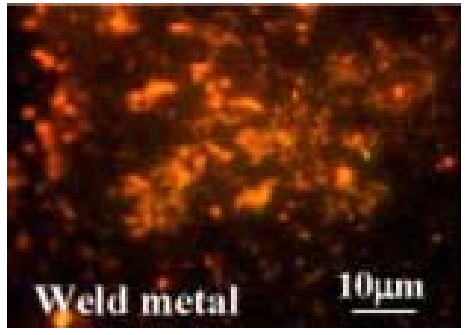


In a laboratory scale simulation test, good interrelation between bacterial attachment and pitting generation was confirmed.

Lab. test : Quantitative analysis of bacterial attachment



As-received



As-weld

- Epifluorescence microscope observation

In a laboratory scale simulation test, weld metal showed much more bacterial attachment than base metal.

Why does bacteria tend to attach on stainless steel welds ?

Stainless steel welds attacked by MIC

In the flow condition……

Water stream

Water stream changes over the bead and stagnant around toe. That is the reason why…….

Amaya et al Corrosion/2002

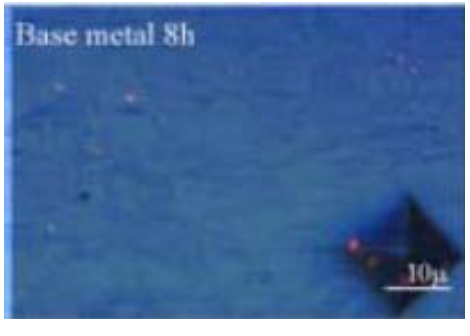
BACKGROUND 4

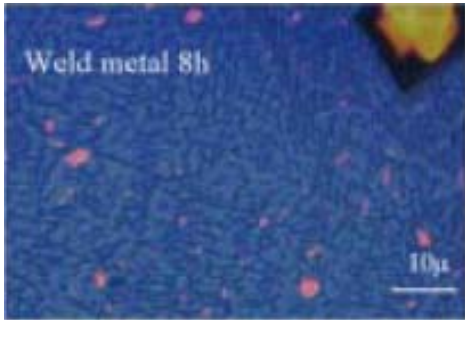
- *The effect of the surface roughness or ruggedness shapes is considered as one of main reasons.*

- *However, even in smooth surface, preferential attachment was detected.*

…and…

- *Preferential attachment on grain boundaries (in base metal) and austenite/ferrite surfaces (in welds) were also confirmed.*





- Superimposed images of 304L SS

K.R. Sreekumari et al Biofouling/2001

VISION

- *This is the indication which shows there exists the interaction of some metallurgical factors and the preferential bacterial colonization on welds.*

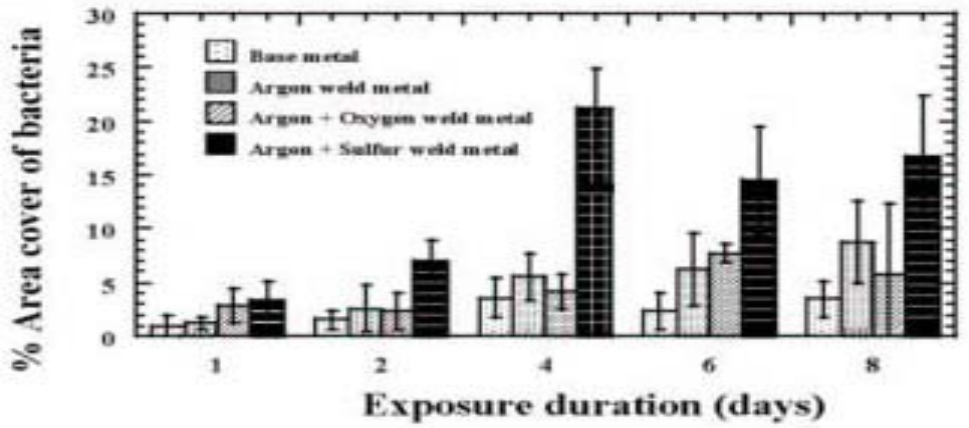
…for example…

- *The difference of surface energy between matrix and boundary*
- *The influence of substratum microstructure or the elemental segregation at the grain boundaries*
  - *Sulfur and Nitrogen are probable elements*
  - *We tried to check this possibility.*

Cf. 1) D. W. Walsh et al. Corrosion/92
2) E. Olsen et al. Corrosion 6
3) K. R. Sreekumari et al. Corrosion/2002

Lab. test : 8 days exposure study using Sulfur rich SS

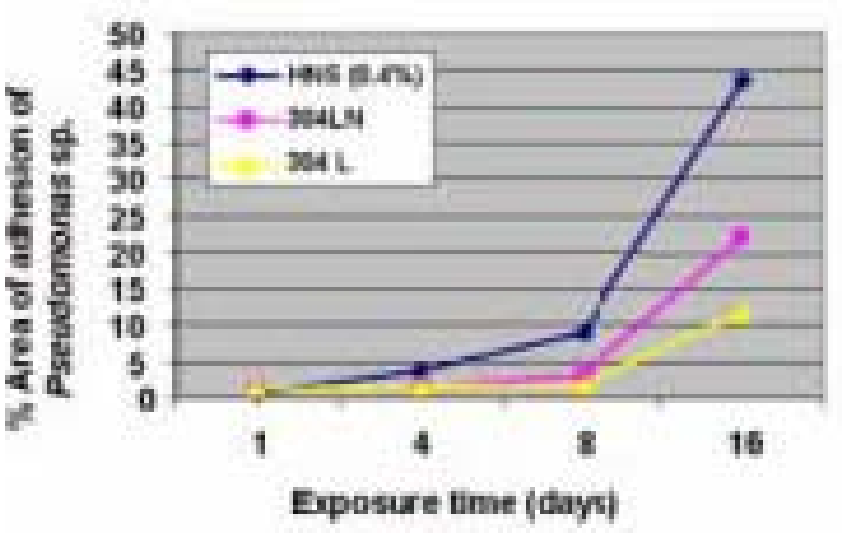
- *Test specimen : Base metal(304L) and welds (S enriched)*
- *Test solution : Pseudomonas* sp. *culture medium*



Sulfur enriched SS welds showed a possibility as a proffered site for bacterial attachment.

Lab. test : 16 days exposure study using HNS

- *High nitrogen containing stainless steel (more than 0.4 weight percent)*
- *Test solution : Pseudomonas* sp. *culture medium*



High nitrogen containing SS (HNS) showed a possibility as a preferred substratum for bacterial attachment.

OBJECTIVE

- The alloying elements, which have possibility to become essence for bacterial growth, enhance bacterial attachment.
- If the elements are profit for bacterial growth, bacterial attachment should be enhanced.
- If the elements are harmful, bacterial attachment should be deterred.

To confirm the efficiency of antibacterial ability of metals for bacterial controlling (avoid attaching) was set as our main object.

EXPERIMENTAL OUTLINE

- ***EXPERIMENT 1***

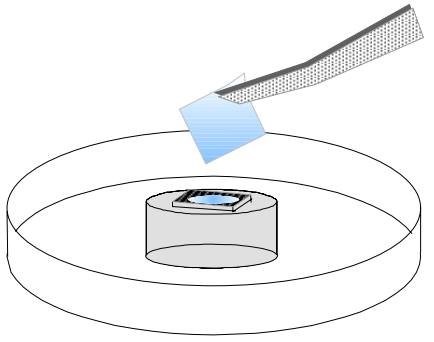
  In order to get a base line data on the antibacterial ability as alloying elements, some metals were tested using a standard method.

- ***EXPERIMENT 2***

  The efficiency of copper and silver, the well-known toxic elements, as an alloying elements in stainless steel were tested in the laboratory and in a freshwater environment .

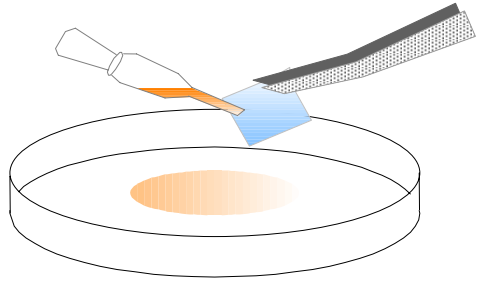
Procedure : Film contact methods based on JIS Z2801

- 75ml bacterial culture liquid were exposed to some metals
- Test sample : Zn, Ni, Pb, Co, Mo, Zr, Cu, Sn and Ti



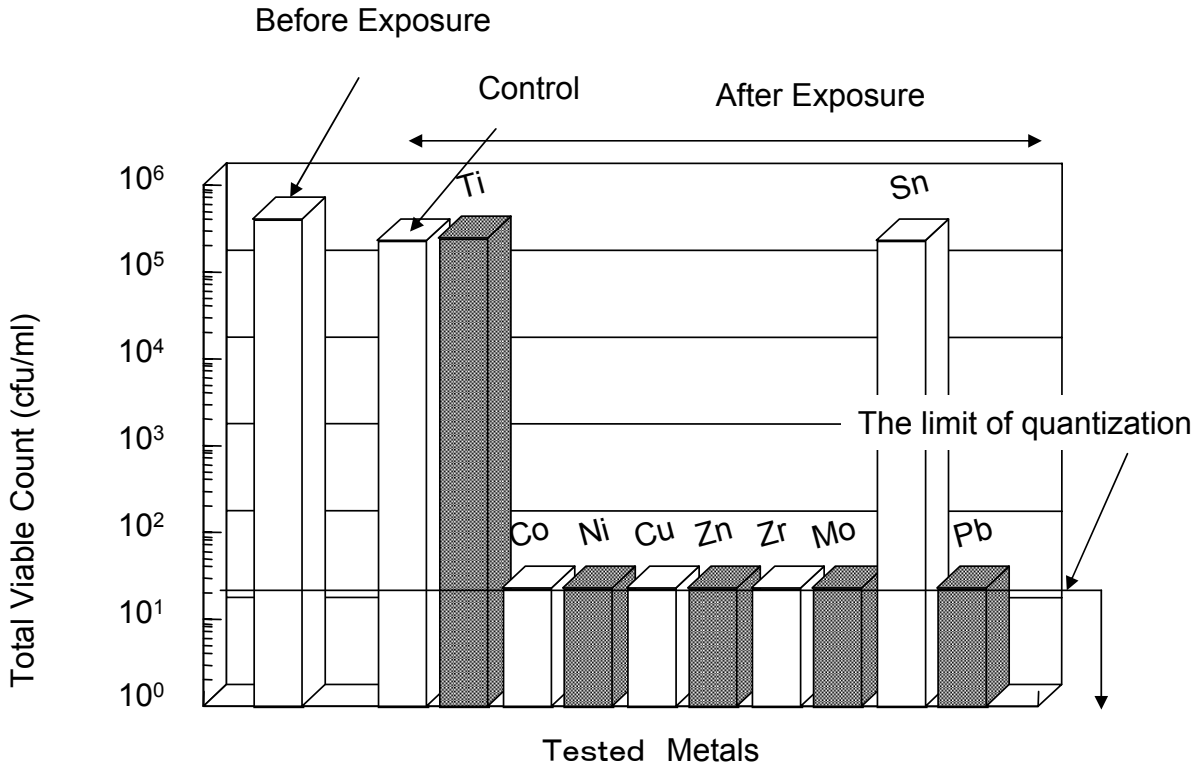
Covered by film

24 hour cultivation at 303K



5ml culture liquid washed from samples were prepared for test

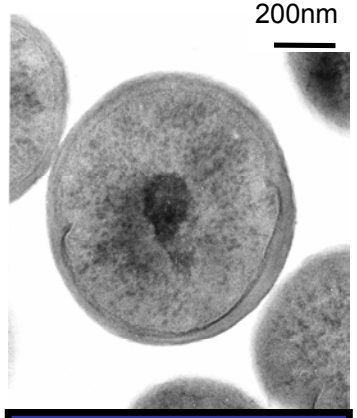
Results: Some pure metals against *Staphylococcus* aureus



Some pure metals showed obvious antibacterial ability by reducing the colony forming units.

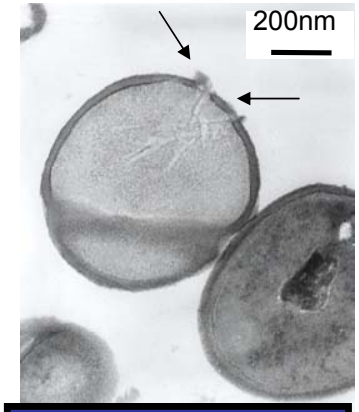
Results : Cell damage observed by TEM after exposure

Control



Sn

Negative



Zn

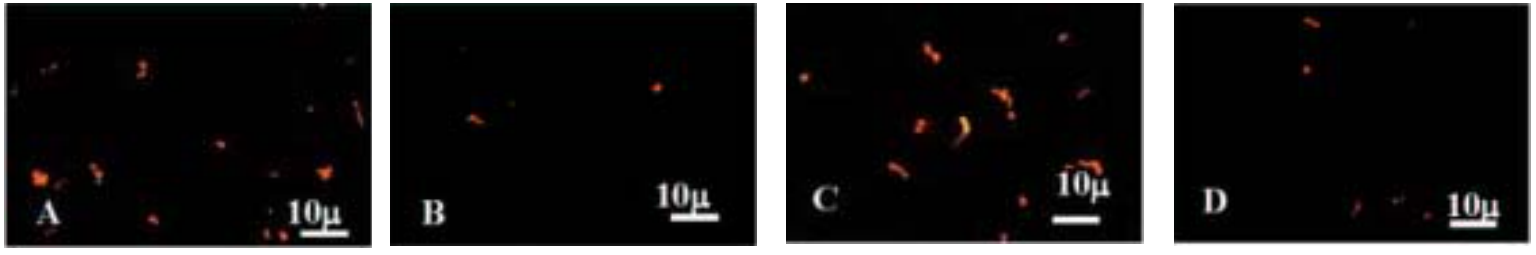
Positive

Significant damage was detected in some pure metals.

EXPERIMENT 2 (Copper contained SS, NISSIN STEEL)

Lab. test : Cu contained SS against *Bacillus* sp.

Base metal | **Weld metal**



AISI 304 | Cu Contained | AISI 304 | Cu Contained

- Epifluorescence microscope observation

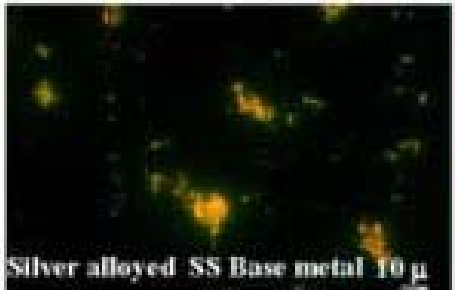
Cu contained SS showed higher resistance to bacterial attachment

EXPERIMENT 2(Silver contained SS, JFE STEEL)

Lab. test :Silver contained SS against *Pseudomonas* sp.

AISI type 304

Silver alloyed (514)



Silver coated

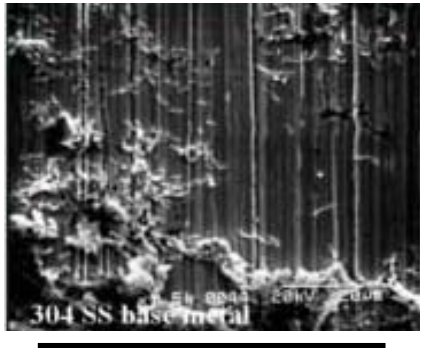
- Epifluorescence microscope observation

Silver contained SS showed higher resistance to bacterial attachment.

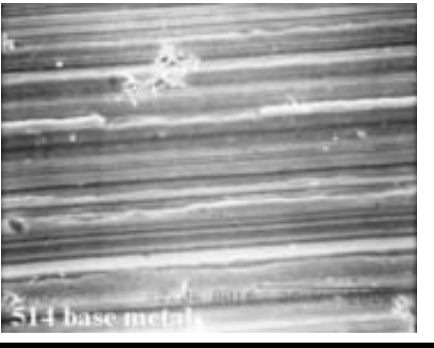
EXPERIMENT 2(Silver contained SS, JFE STEEL)

30 days exposure test to *Pseudomonas* sp. cultibation liquid

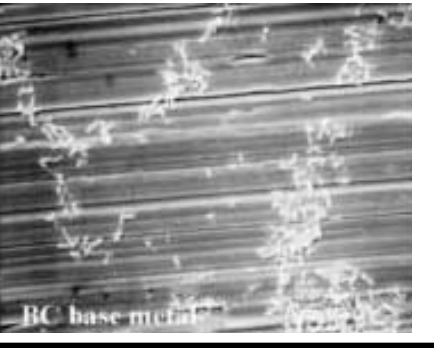
The evaluation for bacterial attachment and pitting generation using SEM



AISI type 304



Silver alloyed (514)

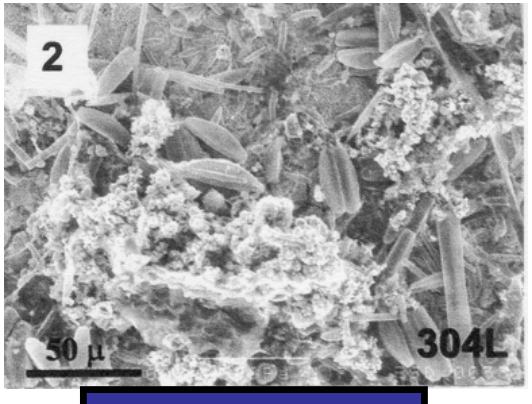


Silver coated

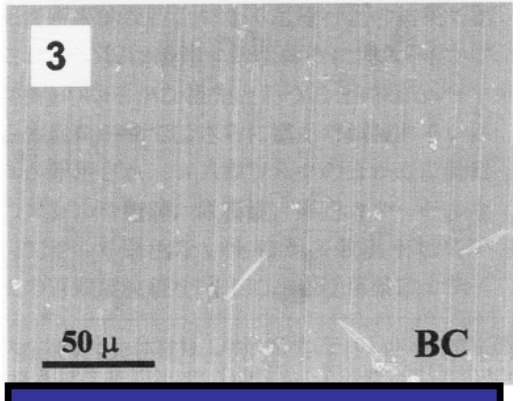
Pitting were generally observed in association with biofilm
Very less numbers of pitting could be observed on Silver contained.
The lowest bacterial attachment and no pitting could be observed on silver alloyed SS.

EXPERIMENT 2(Silver contained SS, JFE STEEL)

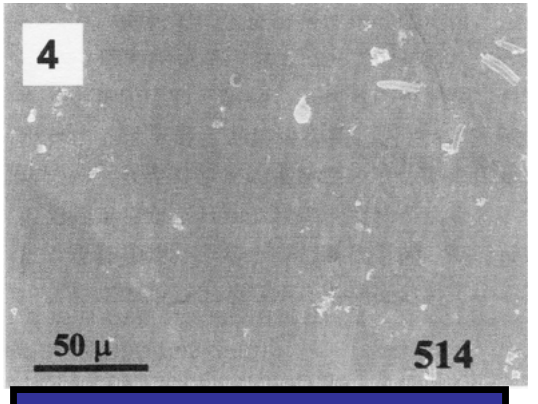
Field test :Silver contained SS in a fresh water



AISI type 304



Silver alloyed (514)



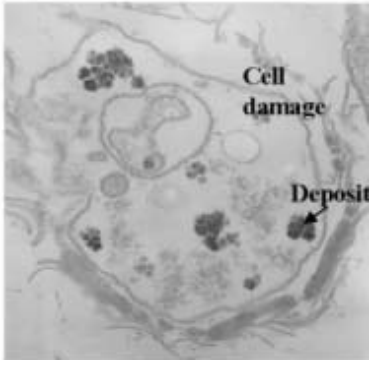
Silver coated

- Environmental SEM images

Silver contained SS showed clean surfaces and was free from diatoms.

EXPERIMENT 2(Silver contained SS, JFE STEEL)

Lab test : The damaged cells (*Pseudomonas* sp.) after exposure



Inside the cell

Over the cell

- TEM observation

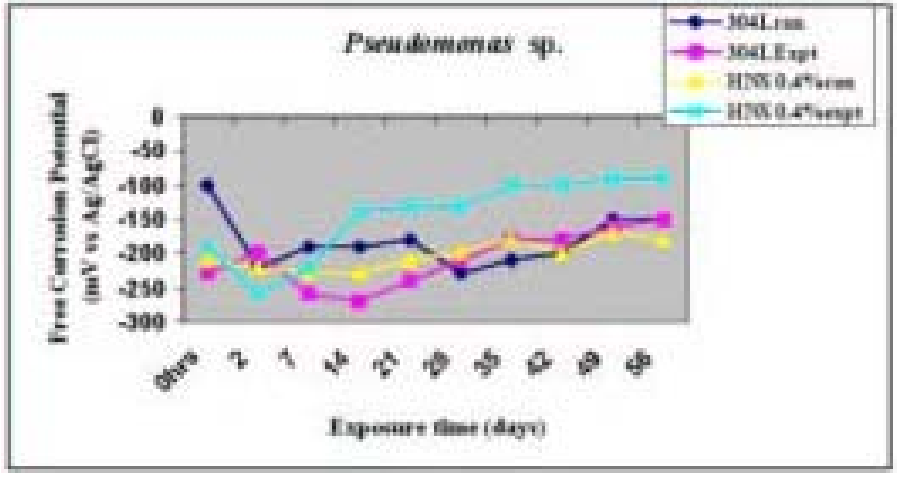
What is the mechanism ?
Still further investigations are needed.
We are assuming the effect of metal ions released from surfaces and the formation of sites resembling metal deposition.

CONCLUSION

- *The study for antibacterial efficacy of some metals and alloys were performed to investigate their suitability as alloying elements to make novel antibacterial metals.*
- *The clear difference of bacterial mortality exposed to some metals and the TEM observations revealed the cell damages after exposure.*
- *The commercial product copper and silver containing SS showed clear effect to mitigate bacterial attachment.*
- *They were resisting micro fouling load to a significant level compared to the normal SS in the field test for a period of 30 days.*
- *The conclusions showed above could form the base line knowledge those could be useful while considering the strategy to control bacterial attachment and MIC.*

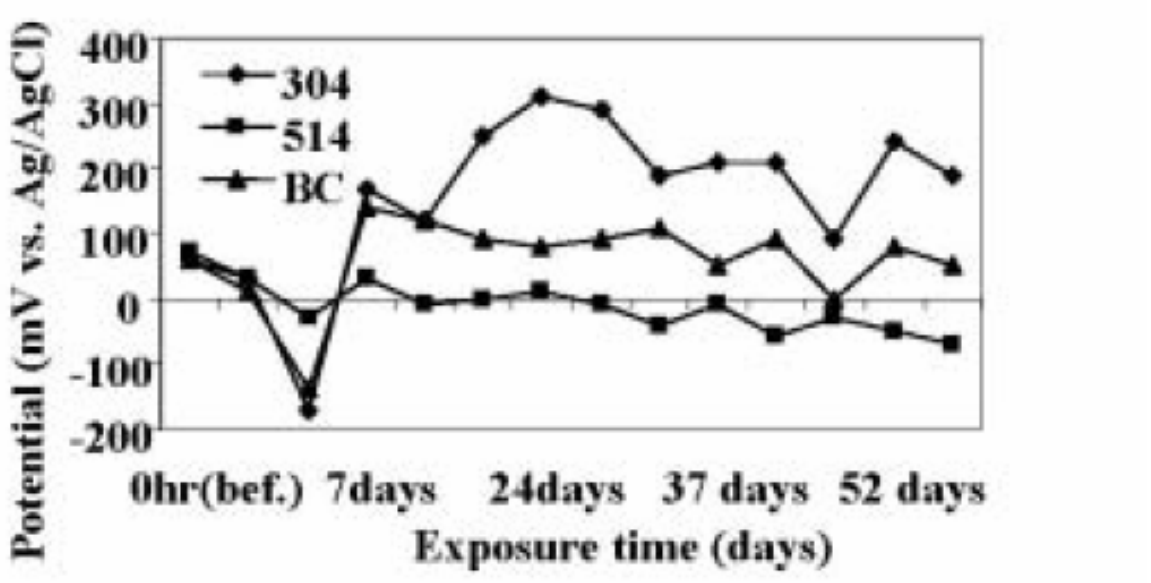
Effect of bacterial attachment on FCP

Free corrosion potential to evaluate a susceptibility of HNS was monitored in *Pseudomonas* sp. culture liquid.



High nitrogen containing stainless steel showed more positive free corrosion potential

60 days exposure test to *Pseudomonas* sp. cultibation liquid



Corrosion probability was monitored in the laboratory using electrochemical technique