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Settlement and growth of copper-tolerant *Ectocarpus siliculosus* (Dillw.) Lyngbye on different copper-based antifouling surfaces under laboratory conditions

Part 1 *Corrosion trials in seawater and development of an algal culture system*

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The first paper of this series reports the development of an algal culture system suitable for monitoring marine antifouling characteristics of copper-based alloy materials under standardized laboratory conditions, using the marine-fouling alga *Ectocarpus siliculosus*. The physical and chemical conditions necessary for both the formation of corrosion films typical of those formed at sea and vigorous growth of the alga in the incubating medium were investigated using copper–nickel 90/10 as a test material. Preincubation of sample plates in seawater for up to 140 days proved necessary in order to generate passive corrosion films which continued to release copper at sufficiently low rates to permit algal growth, spore production and settlement. Seawater sterilization and addition of supplementary algal nutrients did not significantly affect the equilibrium rates of copper loss or the composition of the corrosion films. Aeration of the medium accelerated the attainment of this equilibrium but the addition of Fe^{2+} ions had no effect. Surface preparation and orientation of sample plates had little effect on rates of copper loss. Corrosion rates recorded in these trials compare favourably with those reported for similar materials exposed at sea.

1. General introduction

The biocidal characteristics of copper have enabled it to be used successfully as an antifouling agent in a number of marine applications. As early as the mid-18th Century, copper sheathing was applied to the hulls of wooden seagoing vessels to keep them free of marine organisms which increase friction resulting in loss of speed and manoeuvrability. Major uses of copper alloys where the combination of corrosion resistance and antifouling properties are valuable are in condensers and heat exchangers in ships, power stations and desalination plants and for seawater circulation systems on ships and off-shore oil and gas platforms [1]. Copper in the form of cuprous oxide has been highly successful as the active component in antifouling paints, ridding the hull of hard (shell) fouling although the growth of some macroalgae such as *Ectocarpus siliculosus* can still occur. The development of a coating-based copolymer system with the toxic component tributyl tin has made a substantial advance in reducing algal fouling on moving structures. However, small vessels with copper–nickel hulls have been shown to remain free from fouling in service and it may be economic to apply the same principle to larger

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ships in view of savings in fuel and other costs [2]. Copper–nickel has also been used successfully for the cages of marine fish farms. There is also an increasing requirement for antifouling systems on stationary structures such as the legs of off-shore platforms where copper alloys may again prove both effective and economical.

A small number of organisms are able to survive at copper concentrations in their environment which would be toxic to most other organisms. These copper-tolerant organisms cause some of the most intractable marine-fouling problems. The brown alga *E. siliculosus* has evolved copper-tolerant strains which can grow, albeit slowly, in seawater which is saturated with copper (0.5–1.0 $mg\,l^{-1}$) [3, 4]. This tolerance is brought about in part by excluding copper from the cell and also by internal changes which render innocuous substantial amounts of copper [5, 6]. Copper tolerance accounts for the success of the alga in colonizing surfaces coated with paint containing cuprous oxide.

The growth of *E. siliculosus* on toxic surfaces has not been studied in detail and the resistance that copper-tolerant strains have to different copper-based antifouling surfaces has not been compared. In this study *E. siliculosus* has been employed as a bio-assay organism in controlled fouling trials to compare the toxicity of different copper-based surfaces under laboratory conditions. The vigorous growth of this alga in laboratory culture together with a well-established method of growth assay make it particularly suitable for such trials.

2. Corrosion trials with copper-nickel 90/10

Trials were undertaken to determine the laboratory conditions which were compatible with both the "normal" corrosion of copper alloy surfaces and the healthy growth of *E. siliculosus*. Earlier trials, reported elsewhere [7], indicated that it was not possible to successfully introduce the alga into medium incubating alloy surfaces before formation of a protective corrosion film. Copper losses in the early stages are so great that extreme toxicity results in complete death of the alga. Whilst it is possible to manipulate the copper concentration in the incubating medium by a suitable combination of size of sample plate and volume of medium, it was found that severe growth inhibition always resulted. It was, therefore, deemed necessary to preincubate alloy surfaces to allow corrosion film formation before the alga was introduced into any experimental units. A typical (passive) corrosion film releases copper in only small amounts so that little or no inhibition of growth of the alga should then result before settlement occurs.

Copper–nickel 90/10 was selected as a representative alloy in these trials because (a) it initially exhibits larger, sustained copper losses compared with many other alloy samples on immersion in seawater; (b) its rate of corrosion in exposure in the open sea is known to fall to very low levels, and (c) it is one of the most widely used copper alloys in marine applications.

Appraisal of the experimental conditions used during preincubation had two objectives. The first was to produce corrosion films typical of those formed in the sea and then to ascertain whether or not optimal conditions for the laboratory culture of *E. siliculosus* affected the nature of such a corrosion film. To this end, the following variables were investigated: aeration of the medium during and after corrision film formation; sterilization of seawater; addition of supplementary algal nutrients; surface preparation; "stopping-off" of all edges of sample plates with an inert lacquer and sample orientation within the medium. The second objective was to investigate possible means of accelerating the attainment of constant, low copper losses from the surfaces by aeration and by addition of Fe^{2+} ions to the seawater during incubation.

The copper content of the medium incubating copper–nickel 90/10 samples in a range of laboratory conditions was compared and contrasted with measurements made from specimens which had previously been incubated at sea for 5 months. The full range of combinations of treatments (sample groups) in these trials is given in Table I.

2.1. Experimental procedure

2.1.1. Basic technique

Each sample group comprised replicate plates of copper–nickel 90/10 (50 mm × 50 mm × 2 mm). All plates were freely suspended from the lids of plastic containers by nylon cord (one sample per container) such that they were totally immersed in 2000 cm^3 seawater medium and incubated at room temperature (cf. 20° C). The medium was changed at 14-day intervals when the samples were lightly agitated to disperse loose corrosion product into the spent medium before being placed in fresh medium. The spent medium was made up to its

TABLE I Experimental conditions for incubation of copper–nickel 90/10 samples

Sample group number	1	2	3	4	5	6	7	8	9	10	11	12
Number of replicates	5	5	5	5	5	5	5	2	1	2	5	5
Conditions												
Aeration	–	–	–	–	–	+	+	+	+	+	+	+
Sterilization	–	–	+	+	+	+	+	+	+	–	+	+
Algal nutrients	+	–	+	–	+	+	+	+	+	–	+	+
Fe^{2+} ions	–	–	–	–	+	–	+	–	–	–	–	–
Surface preparation												
Acid-pickled	+	+	+	+	+	+	+	+	+	?	+	+
Machine-abraded	–	–	–	–	–	–	–	–	–	+	–	+
Lacomit application	–	–	–	–	–	–	–	+	+	–	+	+
Preincubation at sea	–	–	–	–	–	–	–	–	–	+	–	–
*Orientation**	vc	vc	vc	vc	vc	vc	vc	vs	h	vs	vc	vc

*v = vertical, h = horizontal, c = corner, s = side.

original volume with distilled water and concentrated nitric acid added to produce a final molarity of 0.014 M. Any precipitate on the side of the container was brought into solution by mixing with compressed air. 30 cm^3 aliquots were removed for copper analysis by atomic absorption spectrophotometry (AAS). The initial trials continued for a total of 140 days.

2.1.2. Aeration of medium during and after corrosion film formation (sample groups 6–12)

Compressed air was bubbled through the medium at a rate of approximately 100 cm^3 min^{-1} using Fleximist air dispersers situated at the bottom of the containers. Once a protective film was formed with low, relatively constant copper losses, two of the five replicates from one sample group were used to ascertain if aeration present during film formation was necessary to maintain these low copper losses.

2.1.3. Sterilization of seawater (sample groups 3–9, 11, 12)

The algal culture medium was based on sterilized natural seawater (collected from near Skegness, Lincolnshire, UK) enriched with inorganic nurients. Bulk sterilization was by autoclaving followed by filtration. One set of samples was also incubated in untreated, unfiltered seawater.

2.1.4. Addition of algal nutrients (sample groups 1, 3, 5–9, 11, 12)

Supplementary nutrients were added to the medium in quantities given in Table II.

2.1.5. Addition of Fe^{2+} ions (sample groups 5, 7)

Solid ferrous sulphate ($FeSO_4 \cdot 7H_2O$ Analar grade) was added to the medium to produce a final concentration of 1 mg l^{-1} Fe^{2+}. Additions were made at each change of medium.

2.1.6. Surface preparation

The copper–nickel 90/10 samples provided by IMI Yorkshire Imperial Ltd received initial surface preparation by deburring and then pickling in acid. After pickling, one set of samples (group 12) was abraded (600 emery). Before use in our trials, holes (1.7 mm diameter) were drilled in one corner of the plates to facilitate suspension and the samples then washed in acetone to degrease surfaces. All edges (to 3 mm), one side and around the hole of some samples (groups 8, 9, 11, 12) were "stopped-off" with three applications of Lacomit (W. Canning & Co Ltd, Sheffield, UK).

Two bars of copper–nickel 90/10, kindly supplied by Mr D. R. Houghton of the Admiralty Marine Technology Establishment, Portsmouth, (sample group 10), had been machine-prepared and immersed in the sea off Oban, Scotland, for

TABLE II Chemical constituents of algal nutrients (modified from von Stosch, 1964)

Nutrient	mg l^{-1}
$NaNO_3$	42
$Na_2HPO_4 \cdot 12H_2O$	11
$FeCl_3 \cdot 6H_2O$	0.27
$MnCl_2 \cdot 4H_2O$	0.0198
KI	1.7
Vitamin B_{12}	0.001

approximately 5 months, then cleaned of superficial corrosion product and stored dry.

2.1.7. Orientation of the test surface

Lacomit was applied to two replicate samples as described above. The plates were suspended vertically from the centre of one side from perspex rods glued to the Lacomit side (sample group 8). One sample (group 9) was similarly prepared and mounted horizontally, experimental surface downwards.

2.2. Results (Fig. 1)

Initial copper losses from all samples were in excess of $30\,\mu g\,cm^{-2}\ day^{-1}$. During the first 12 weeks of incubation these losses gradually decreased to a constant value of approximately $2\,\mu g\,cm^{-2}\ day^{-1}$ suggesting that protective corrosion films had then formed. There was some variation in the time taken to reach this equilibrium state but none of the incubation conditions investigated led to a wide deviation from the above pattern of copper loss during corrosion film formation. Once protective films had formed, all samples lost $1.3–2.2\,\mu g\ cm^{-2}\ day^{-1}$. This copper loss was similar to that from the bars of copper–nickel 90/10 incubated in our laboratory conditions after formation of a typical corrosion film at sea ($1.6–2.8\,\mu g\,cm^{-2}\ day^{-1}$).

Initial copper losses were greater from samples in aerated media than in the unaerated media but the rate soon diminished and equilibrium was reached more quickly (6 weeks rather than 10 weeks); rates at equilibrium were, however, very similar (Fig. 1a). Sterilization of the seawater medium by autoclaving caused a slight increase in rate of copper loss initially, and a more rapid reduction in rate similar to the effect of aeration (Fig. 1b). These differences, however, did not prove to be statistically significant. Addition of algal nutrients did not influence the copper losses from the samples during preincubation (Fig. 1b). The presence of $1\ mg\ l^{-1}\ Fe^{2+}$ ions replenished at bi-weekly intervals did not change the rate of copper loss from the sample surfaces (Fig. 1a). Copper losses from machine-abraded surfaces were similar to those from samples which had been acid-pickled only and losses from samples with the edges and one side coated with Lacomit were greater at equilibrium compared with uncoated samples (Fig. 1c). However, visual inspection showed that the corrosion film formed on the uncoated samples was less uniform with "streaming" of corrosion product from the suspension holes. In the simple trials reported here, the orientation of the plates within the incubating medium and the type of suspension used had no detectable effect on copper losses from the surfaces (Fig. 1d).

3. The experimental system

3.1. The bio-assay organism

Ectocarpus siliculosus (Dillw.) Lyngbye (Phaeophyceae) is a filamentous, branched brown alga, reproducing largely asexually by means of zoospores liberated from sporangia borne terminally on fertile filaments. These motile zoospores can settle on a substrate or another organism to give rise to a plant comprising two distinct parts: prostrate and erect filaments. On the hulls of ships adult plants may produce a continuous covering referred to as a "brown mat". Alternatively, germination of zoospores and subsequent development of sporelings can give rise to free-floating filamentous masses.

3.2. Source and culture of the alga

The copper-tolerant population of *E. siliculosus* used in this study was obtained from the collection of O. P. Morris who isolated it from samples collected by scraping the hull of the seagoing vessel "San Nicholaos" which had been painted with copper-based antifouling paint. The isolate was demonstrated to have a high tolerance to copper [3, 4]. A unialgal stock culture was maintained in sterilized, enriched seawater medium in glass beakers in a Gallenkamp incubator at $15° \pm 1°$ C and continuous illumination of 1000 lux. The growth medium was replaced monthly and the alga regularly sub-cultured.

3.3. Algal growth in the presence of filmed copper–nickel 90/10 surfaces

The corrosion trials reported in Section 2 suggested that by day 140 protective films had formed on sample plates and that films produced in sterilized, aerated seawater enriched with nutrients were not atypical in any way. Incubation of filmed plates from sample group 6 was continued to allow the introduction of the alga. Approximately 50 mg wet weight of alga were added to the medium incubating two replicates and the aeration to one unit was discontinued. Two units similarly treated but lacking the alga served as controls. All units were illuminated with a bench light in the same experimental conditions as before. Incubation

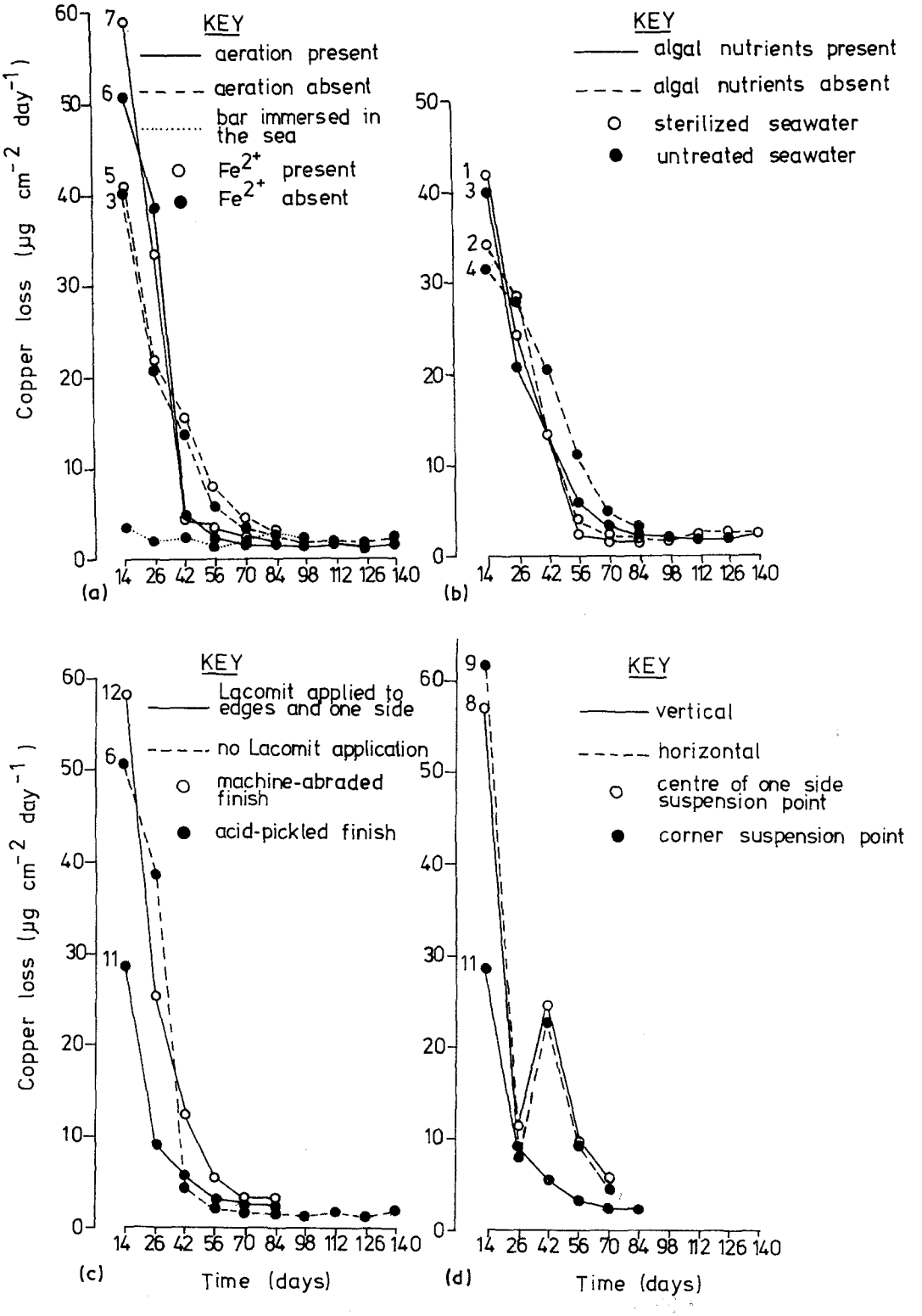

Figure 1 Copper losses from copper–nickel 90/10 samples incubated in static seawater under laboratory conditions for 140 days. The medium was replaced at each 14-day sampling interval. Preparation of samples, numbers of replicates and experimental conditions are given in Table I. Numbers beside each curve refer to sample groups arranged to illustrate the effects of: (a) aeration of medium and addition of Fe^{2+} ions; (b) seawater sterilization and addition of supplementary algal nutrients; (c) preparation of sample surface; and (d) orientation of sample plates.

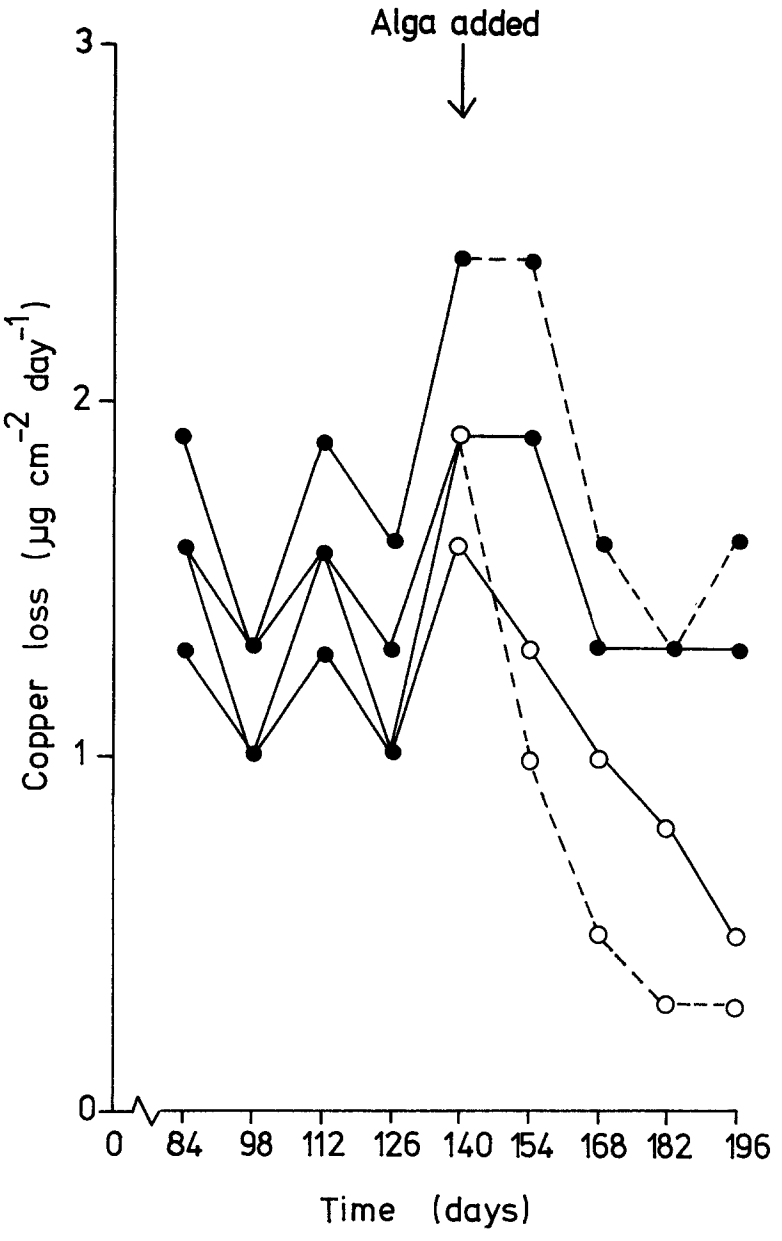

Figure 2 Copper losses from four copper–nickel 90/10 samples incubated in static seawater for 196 days. At day 140 aeration of two units was terminated and an inoculum of *Ectocarpus* added to two units, with and without aeration. Further details of sample preparation and experimental conditions are given in Table I. – Aeration present; – – – aeration absent; ○ *E. siliculosus* present; • *E. siliculosus* absent.

proceeded for a further period of 56 days, with medium changes at 14-day intervals.

It was soon apparent that normal growth and reproduction of the alga was possible under the conditions prevailing. Rates of growth were initially slow as copper concentrations in the medium at the end of the preincubation period (day 140) were clearly still high enough (> 0.5 mg l^{-1}) to cause some growth inhibition. However, the growth rate of the alga then increased rapidly as the copper content of the medium was reduced (Fig. 2). This reduction in ambient copper concentration was due to copper uptake by the alga itself when sufficient biomass had accumulated. After 6 weeks incubation it was clear that the growth of the free-floating masses of alga was healthy as was evidenced by the presence of spores in the medium. There was, however, some slight reduction in copper losses from the preincubated plates in the absence of the alga, but this was within the range of fluctuations observed to have occurred in the latter stages of the preincubation period. In the presence of the alga, apparent rates of copper loss fell to below 0.5 μg cm^{-2} day^{-1} by day 196. Equilibrium rates in the absence of the alga were about 1.5 μg cm^{-2} day^{-1}.

TABLE III Metal distribution in the corrosion product of four samples of copper–nickel 90/10 incubated under different conditions. The atomic percentage given has been calculated from the area under peaks from XPS analysis

Aeration	Conditions of incubation			Surface	Atomic percentage of metal			
	Sterilization	Nutrients	Fe^{2+} ions		Cu^{+}	Cu^{2+}	Fe	Ni
				A	28.67	52.23	5.22	13.58
–	–	+	–	B	50.56	38.95	7.17	3.82
				C	38.23	39.80	11.95	10.00
–	+	–	–	A	20.51	66.11	13.38	–
				C	27.32	59.10	4.34	9.25
				A	22.61	56.28	21.11	–
–	+	+	+	B	2.81	97.19	–	–
				C	73.47	12.94	13.58	–
+	+	+	+	A	22.68	67.04	10.28	–
				C	63.06	31.56	5.38	–

Three surfaces were examined where possible: A is the outer surface of corrosion product; B is the inner surface of corrosion product; and C is the alloy surface after removal of corrosion product.

4. Discussion

4.1. Formation of a corrosion film under laboratory conditions

Corrosion films formed on the copper– nickel test plates in the range of preincubation conditions employed were examined quantitatively by X-ray photo-electron spectroscopy (XPS). A summary of these analyses is given in Table III for different surfaces of the corrosion product. While it is apparent that different preincubation conditions did produce corrosion films of slightly different composition, none clearly resulted in any extreme modification. All corrosion films formed after 12 weeks were regarded as chemically similar to those observed on operating plants and test rigs at sea (private communication J. E. Castle).

Copper losses from corrosion films formed under laboratory incubation can be compared with published data for weight loss from samples of copper–nickel 90/10 exposed in corrosion trials at sea. The average copper release rates over 140 days for unaerated and aerated media (9.0 and 11.0 $\mu g\ cm^{-2}\ day^{-1}$ respectively) were similar to those reported by Efird and Anderson [8]: 10.0 $\mu g\ cm^{-2}\ day^{-1}$, and by LaQue [9]: 7.0 $\mu g\ cm^{-2}\ day^{-1}$ for samples exposed in quiet water for one year.

Furthermore, the fact that rates of copper loss from films formed in the laboratory-aged samples were similar to those from bars aged at sea and then transferred to laboratory conditions, suggests that films formed under these two different sets of conditions subsequently behave in a similar manner when subjected to standardized incubation.

Of the conditions investigated, only one: aeration, significantly changed the rate of copper loss from samples during 140 days incubation. Aeration accelerated the formation of a protective film. LaQue [9] has also suggested that aeration facilitates film formation on copper–nickel alloys. Visual examination of sample plates confirmed that those generated in aerated media were also more uniform in appearance.

The presence of Fe^{2+} ions, added intermittently did not reduce the corrosion rate as has been found with seawater-cooled condensers and heat exchangers [1]. This is probably because protective film formation is enhanced only by flowing seawater and regular Fe^{2+} ion addition. The time taken for protective film formation was also unaffected. Differences in sample surface preparation and different orientation of plates during incubation had little effect on final corrosion rates. However, visible irregularities of the surface of some samples suggested that a machine-finished surface with Lacomit applied to edges and one side, suspended vertically in the medium produced the most uniform corrosion film.

4.2. Algal culture and its effect on film formation

In order to produce vigorous unialgal cultures of *Ectocarpus* it is necessary to sterilize seawater to remove all other algal and bacterial contaminants and to enrich with supplementary nutrients to stimulate growth. It was found that both sterilization of seawater and addition of supplementary nutrients were compatible with typical corrosion film formation. As corrosion rates were not

influenced it must be concluded that any resulting changes in the chemical compostion of seawater were not important in the corrosion processes.

4.3. Incubation of *Ectocarpus* in the presence of filmed alloy surfaces

The trials reported in this paper indicated that extreme toxicity problems could largely be overcome by preincubating sample plates until protective corrosion films had formed. However, even with the incubation system described, copper concentrations at the end of the preincubation period were still high enough ($> 0.5\,mg\,l^{-1}$) to bring about some inhibition in the early stages of growth of the alga. Copper concentrations in the medium are subsequently reduced by uptake into the alga itself once it has established and produced sufficient biomass for this reduction to be significant. The process then becomes autocatalyic. The initial stages of growth upon introduction into the medium are thus critical. In view of this potential problem, a further modification was incorporated in the final bio-assay system. Instead of introducing a standard weight of algal inoculum into the vessels incubating filmed sample plates, the alga was introduced into vessels containing medium only for 20 days prior to the immersion of the filmed plates. In this way, the walls of the vessels became extensively colonized by the alga which, when the plates were introduced, was thus already growing vigorously. Much of the copper released from the corrosion films would be taken up rapidly by the actively growing algal filaments so that potentially toxic concentrations, inhibitory to spore formation and release, do not build up in the medium.

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