

# On harmonic generation in nonlinear biological systems

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In a recent paper in this journal, Hutchings, Blake-Coleman & Silley (1994) reported some measurements in which they applied a sinusoidal constant-current source to a suspension of *S. cerevisiae* in a dual-cell configuration (a second electrochemical cell contained supernatant) and attempted to measure whether the micro-organisms exhibited nonlinearities, as judged by the differential generation of harmonics. They reported that “no significant *repeatable* harmonics were observed at frequencies from 5 to 80 Hz”. This led them to “focus on the failure to repeat earlier published results, in particular the recent studies by Woodward & Kell (1990, 1991)” and to state that “the observed harmonics (in the Woodward & Kell work) were subsequently, (we feel prematurely), attributed to the effect of membrane enzymes coupling electric field energy discontinuously to normal catalytic cycles”. Hutchings *et al.* then stated that the Woodward and Kell approach “suffers from two serious systematic flaws”, one concerning phase information (which is not recorded in the power spectra shown by us, since we have never in fact found that phase information was of benefit in the systems we have studied), and a second concerning an inadequate subtraction of cellular nonlinearities from those generated by the electrode/electrolyte interfaces in supernatants of suitable conductivity taken from the suspensions under study. They believed that the generation of harmonics that they observed in a somewhat different configuration, was due to a modification

by the biological cells at the electrochemical interface of the behaviour of the electrode/electrolyte interfaces. Hutchings *et al.* concluded “We believe this work has established that there is minimal, if any, significant harmonic generation in the bulk of the suspensions of the organisms tested thus far” and that “. . . the variance of this from other published work (Woodward & Kell, 1990/1a) is shown to be due principally to electrode-generated nonlinearities affecting the bulk current flow”.

We wish here in this brief note merely to point out that the inability of Hutchings *et al.* to reproduce our findings is very simply explained on the basis that they used a different experimental system from us, so it is hardly surprising that they obtained different data. In particular, they used a constant-current and not a floating source, which tends to have the effect of removing the observable nonlinear dielectricity. In our yeast work, the base spectra of which we have reproduced hundreds of times, the harmonics were cell concentration-dependent, not seen in boiled cells, were compared with the correct supernatants, and were abolished by vanadate, a specific inhibitor of the H<sup>+</sup>-ATPase of the organisms (Woodward & Kell, 1990) unless a strain with a specifically vanadate-resistant ATPase was used (Woodward & Kell, 1991b). These are all powerful controls, which strongly support the interpretation that the major source of differential nonlinear dielectricity is the H<sup>+</sup>-ATPase of the intact organisms. Other work on

different biological systems which was published previously and relevant to this subject (not considered by Hutchings *et al.*, however), showed that appropriate and specific respiratory chain inhibitors abolished the nonlinear response in heterotrophic (Woodward & Kell, 1991c) and photosynthetic (McShea *et al.*, 1992) bacteria. None of the inhibitors used was electroactive. We would comment that any criticism of a scientific project that any authors wish to make should take into account all the data which were actually obtained, and especially the many controls mentioned above that were run. Given in particular that the generation of nonlinear dielectricity in these bulk cell suspensions has a well-developed theoretical foundation (Kell *et al.*, 1988; Westerhoff *et al.*, 1988; Astumian & Robertson, 1989; Davey & Kell, 1990) it should be obvious that whatever their own difficulties, Hutchings *et al.* displayed an extremely selective analysis and reporting of our own findings and our numerous controls.

In a later paper, the same group (Blake-Coleman *et al.*, 1994) report "preliminary results of the harmonic analysis of microbial suspensions" (we do not know whether preliminary now means "repeatable"), and amplify their claim (despite the fact that an electrode double layer is only a few molecules thick), that "the characteristic interfacial nonlinearity is viewed as variable, being uniquely modulated by the presence of particular cells". Purportedly in support of this, an experiment is displayed (Fig. 2 of that paper) in which a concentrated suspension of 0.4  $\mu\text{m}$  diameter hollow polymer spheres are added to a KCl solution and the frequency-dependent linear impedance observed to differ from a solution in which the polymer spheres are absent. This is deemed to confirm "that the impedance of the electrode interfacial region is influenced significantly by the presence of proximate, dielectric particles" but could as easily be interpreted on the basis of the observably greater bulk impedance when the spheres are present, (since, all else being equal, electrode polarisation impedances are widely observed to increase as the bulk conductance [or current density in a constant-current system] increases; see for example Kell, 1987; Davey *et al.*, 1990; Kell & Davey, 1990; Oskam *et al.*, 1991; Schwan, 1992; Beving *et al.*, 1994). When the same system was tested for harmonic generation, an appropriate control was not shown (it being necessary to compare the

systems with the same bulk solution properties and conductivity but with and without the polymer spheres). One sensible general control is hinted at in the discussion (the use of a, presumably dialysis, membrane which would keep the cells away from the electrodes) but dismissed. (We can report that in our system such controls lead to exactly the same nonlinear dielectric response, providing further evidence for the irrelevance of any cell/electrode interaction in our system.) When planktonic cells at reasonable concentrations affect electrochemical interfaces, it is overwhelmingly via the uptake and excretion of molecules which leads to changes in the medium adjacent to the electrodes, and thus indirectly to changes in electrode polarisation (Harris & Kell, 1985; Kell, 1987; Kell & Davey, 1990), and hence in the (linear and nonlinear) interfacial electrochemical properties (Woodward & Kell, 1991a). Any future re-evaluation of the influence of micro-organisms on nonlinear electrode processes would beneficially take this into account.

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