

Preliminary Investigation into the destruction of biological material in aqueous suspensions using radiofrequency waves

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Executive Summary

An apparatus was constructed whereby radiofrequency (RF) radiation could be applied to a suspension of yeast and contained in a plastic sample tube. The source of irradiation was a sleeve of plastic tubing around which 4 coils of wire were attached linked to an RF generator. The sleeve fitted snugly around the outside of the sample tube.

The aim of this project was to monitor any effects produced by the RF field upon the suspended material. The major conclusion that can be drawn from this work and using the experimental set-up described as above is:

1. There is evidence that RF radiation has an effect on the adhesion of yeast cells to a surface. The irradiation also resulted in fewer clusters of cells and more single separated cells in suspension.

Our conclusion from these studies is that there is sufficient evidence that the effect of RF waves on yeast in beer and associated products is adverse, preventing the cells from clustering and reducing the build up of yeast .

1. Introduction

The Sonochemistry Centre at Coventry University has a background of research into many different aspects of the effects of ultrasound in chemistry and processing.

Among these are:

- Chemical synthesis
- Electrochemistry
- Food technology
- Fundamental studies of acoustics
- Material processing
- Materials extraction
- Reactor design and modeling
- Therapeutic ultrasound
- Water treatment

It is the interest in the chemical and biological remediation of yeast in beer that has led us to embark on this project involving a preliminary laboratory assessment of the use of radiofrequency waves in the treatment of beer and lager.

2. Ultrasound in water treatment

Research into the use of ultrasound in environmental protection has received a considerable amount of attention with the majority of investigations focusing on the harnessing of cavitation effects for the destruction of biological and chemical pollutants in water. One of the major advantages of ultrasound in this context is that, at the appropriate frequency and power levels, it can be used alone for treatment. A second advantage is that lower levels of acoustic energy can be used to enhance existing treatment regimes e.g. chlorination. A number of presentations and research papers have been generated from the group and large pool of background expertise and knowledge on methods of monitoring water (and other liquid) disinfection has accrued over years [1-4].

The Sonochemistry Group in Coventry University has established a reputation for assessment of the effects of ultrasound on liquids. The methodology for this has been tried and tested over many years. With this background in mind it seemed eminently reasonable to embark upon an analysis of the effects of radiofrequency on similar suspensions.

The team assembled for this project consisted of Professor Tim Mason (director of the Sonochemistry Centre), Dr. Nigel Parker (microbiologist) and Delwar Hussain (recent graduate in Pharmaceutical Sciences).

3. Experimental set-up

It was the view of the team that it would be impossible to mimic precisely the conditions pertaining to the installation of the StayClean System in a working cellar's beerlines. It was necessary to construct a system that could not only be used in the laboratory but also cleaned (i.e. sterilised) regularly. To this end four radiofrequency coils were wound onto a 20 inch long sleeve of plastic tubing internal diameter 1 inch. The sample of yeast (*Candida utilis*) was then placed in a plastic tube of just under 1 inch diameter that served as the reaction vessel. This tubing could then be readily demounted and sterilized as required.

Suspensions of a yeast (*Candida utilis*) in a normal saline were exposed to radiowaves in a static (non-circulating) apparatus. Microbial suspensions were contained in the inner plastic tube (45ml volume) which was then inserted into the outer tube wrapper with 4 coils. The radiowave emitted cycled systematically across a range of frequencies, and its activity was confirmed by the use of a radio receiver that emitted an audible "buzz" in close proximity to the coils.

The effects of short and long term periods of exposure were studied by assessing:

- Microbial viability, under normal and enhanced pressure, was assessed through the determination of viable counts. (Samples were taken periodically from the bottom of the inner tube, or from three sections within the tube).
- Adhesion to surfaces, and the physical arrangement of cells in suspension (aggregation) was studied using microscopical examination.

Throughout this report the term "untreated" signifies the results obtained from microbial suspensions contained within tubing but not contained within the radiowave producing coils (i.e. these were the control measurements). On the other hand "treated" data are those obtained from samples in tubing placed within the effects of radiowaves. All of the results were obtained at room temperature.

4. Results

Effects of radiowaves exposure on microbial adhesion to surfaces

These investigations were devised to assess if radiowaves affected any adhesion of micro-organisms to a plastic surface.

Microbial suspensions, contained in 4 Leighton tissue culture tubes, were placed horizontally inside the outer tubes encircled with coils. At hourly intervals of continuous exposure to radiowaves an individual tube was removed. The arrangement of the cells in the suspension was observed. Tubes were washed twice and the patterns of adherent cells on the cells on the tube surface determined.

Experiments using yeast showed that when exposing the cultures to radiowave using the radiowave emitter there were few clusters of cells and more single separated cells, in comparison to not using the radiowave emitter. There appeared to be a reduction in the formation of cell clumps in suspension. Adherent cells were less clustered or clumped in radiowave treated samples.

Time (h)	Untreated	Treated
0	Lots of cells in single and clustered form	Lots of cells in single and clustered form
1	Long chains of clustered cells separating into some single cells	Small clusters of cells and some single cells scattered
2	Long chain of cells with few single cells	Evenly spread single cells with small clusters
3	Few large clusters with some single cells	Lots of evenly spread single cells
4	Thin long strands of cells, and some single cells	Some clusters of cells and many single cells
24	Lots of cells, all aggregated together.	Very difficult to see, very small, cells aggregated together with single cells which are very difficult to distinguish.

5. Discussion

Some effects on adhesion were shown. Direct observation showed that exposure to radiowaves appeared to have an effect on adhesion by yeast. Exposing the cultures to radiowaves resulted in fewer clusters of cells and more single separated cells. This suggests the radiowaves are reducing the formation of, or are causing the dispersion of, microbial clumps within the tube. It is suggested therefore that the mode of action on radiowaves is to affect the physical organisation of microbes in suspension and that this reduces adhesion to a surface. This is the reason why beer lines require less cleaning whilst they are being treated with the radiowave emitted known commercially as the StayClean Beerpipeline System.