Monitoring Methanogenic Fluorescence by Image Analysis

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The presence of the co-factor F_{420} gives to the methanogenic bacteria the specific ability of auto-fluorescence when excited at a wavelenght of 420 nm. The F_{420} is found in other bacteria such as *Streptomyces*, but at levels far below those found in methanogenic bacteria [1]. Therefore, in pratice, it is possible to use the blue-green autofluorescence to differentiate between methanogenic and non-methanogenic bacteria [2]. However, the analysis of the intracellular F_{420} is not a suitable routine method due to its complexity and the lack of commercially available F_{420} molecule. Furthermore, the usefulness of this property in the identification of different genera of methanogenic bacteria is not evident and the great variability of F_{420} levels in the different genera of methanogenic bacteria, along with the sensitivity to environmental conditions, makes difficult the interpretation of experimental data. Due to the above mentioned problems, the very high initial expectations in the use of intracellular F_{420} analysis as a tool to measure the methanogenic activity were partly frustrated. However, the intensity of the blue-green emitted light after excitaton at 420 nm is potentially useful to predict the overal methanogenic activity. In general, hidrogenotrophic bacteria present higher levels of F_{420} than acetoclastic bacteria. For instance, *Methanosaeta* (ex *Methanothrix*) possess only residual levels of F_{420} [3].

Image analysis was used in this work to quantify the blue-green light intensity developed during the start-up of a CSTR fed with a VFA based synthetic substrate and during the steady state operation of an anaerobic filter fed with a synthetic dairy waste. The possibility of release of F_{420} to the medium keeping its blue green fluorescence was considered by discounting the backgroug blue green intensity in order to eliminate unrelevant information (originated probably from dead cells). The images were acquired by microphotography and subsequently digitised to a 24 bit (true color) JPEG format by a HP Deskscan. A program was written in MATLAB 5.1 (The Mathworks, Inc.), to calculate the number of bacterial cells and its fluorescence intensity. The image was split in Red (R), Green (G) and Blue (B) components. After concluding that the B channel originated a greater distinction between the dark background and the fluorescent cells, all the subsequent images were processed in the B channel only. The following operation consisted on achieving a mask of the objects brigther than a pre-determined threshold (the value of 0.88 was found to be adequate for choosing only the brighter cells). The background was then computed and subtracted from the image which compensated for illumination differences in the image. The backgroung was calculeted by extracting the darker pixel in 4x4 squares into a 128x128 image and retransforming it with the bicubic extrapolation method. A new mask was obtained from this image with the pre-set threshold 0.85. Cells in a bright background were poorly recognised by this mask, but the first mask was very useful in recognising those cells. Therefore the cells could be recognised using both masks by performing a logical *or* operation and a cell, to be considered as one, has to appear in at least, one of the two masks.

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