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Application of an image analysis system for quantification and classification of environmental microbes from agricultural biogas plants

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The goal of this study was to find a new tool to evaluate the biological vitality of a biogas plant. Therefore, a simple microscopic method for quantification and morphological classification of microorganisms was developed. Cell counts and its morphology are supposed to be a direct indicator for the performance of a methanogenic habitate [1]. Since environmental samples contain interfering fibrous material, an appropriate sample preparation is required such as an optimal visualizing stain, homogenous distribution of sample on the slide as well as ~~a~~ proper image analysis algorithms. SYBR Green I was found to be the most sensitive dye for total cell counts in environmental samples with minimal background fluorescence [2]. For detection of methanogens, auto-fluorescence based on the typical methanogenic fluorescent coenzyme F_{420} was used. A low viscosity methylcellulose was successfully applied as a fixing agent for microscopic slide. It showed better adhering character and supplied more regular surface related to agar or gelatin. With the help of accompanying molecular analysis [3], morphological classification algorithms for methanogens could be established by the image analysis software Image Pro 7. During analyzing one biogas plant for more than 26 weeks and several different anaerobic digesters for a shorter time, its reliability as a counting method was verified.

The correctness of the counting by image analysis system was proven by Neubauer counting chamber with microbial cells like *Staphylococcus* and *Escherichia coli.* Furthermore, interfering particles were tested by inorganic nano particles, maize fibres and artificial fluorescent beads. The results showed a good congruence between quantitative image analysis and Neubauer counting chamber representing a deviation of 0.2 – 2.6 %.

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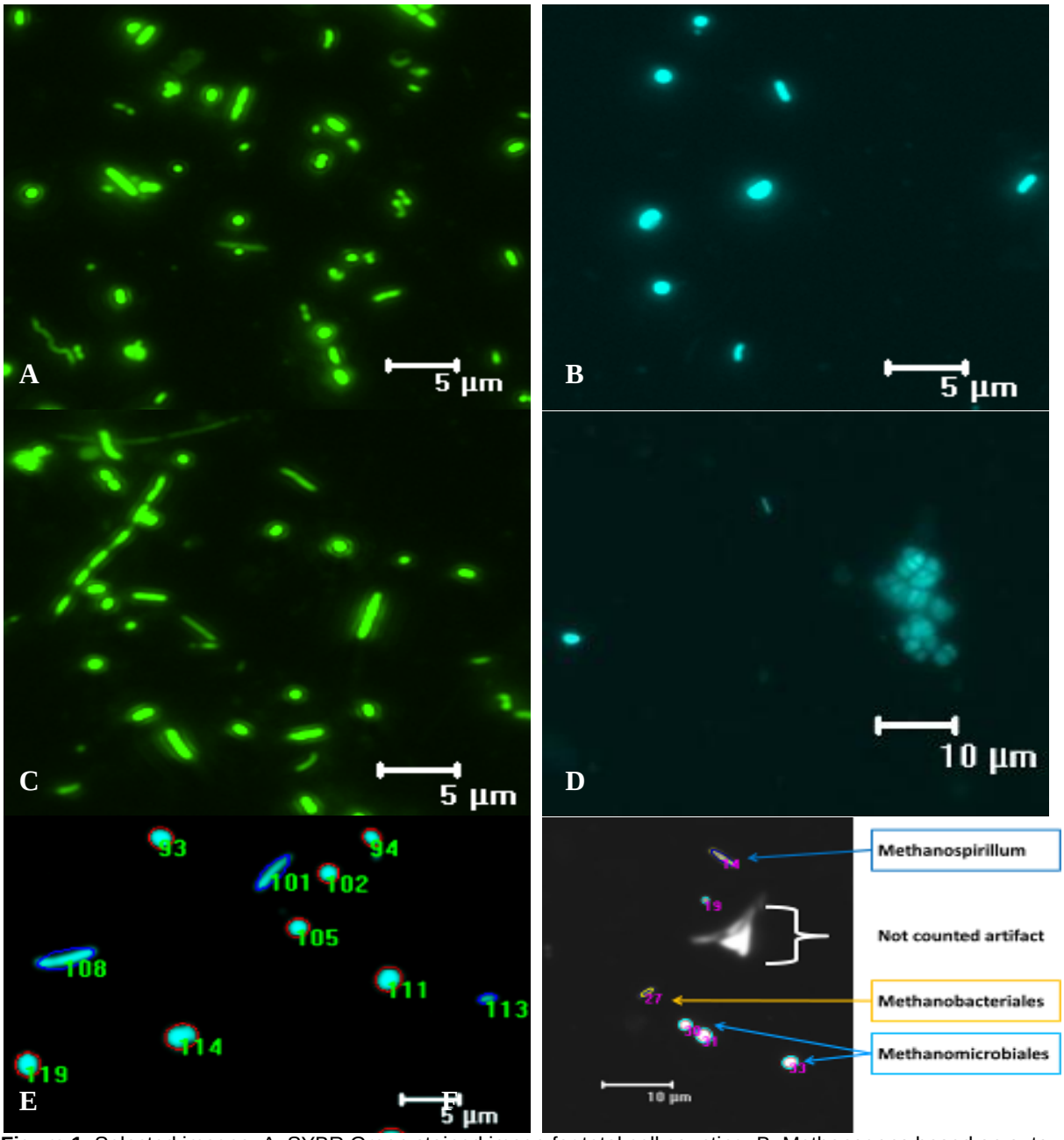

Figure 1. Selected images: A: SYBR Green stained image for total cell counting; B: Methanogens based on auto-fluorescence; C: Long and sheated filament of *Methanosaeta (left) being surrounded by different morphotypes*; D: Cellular packets of *Methanosarcina*; E, F: Automatically counted methanogens with different coloured circles around them. They were classified and quantified by developed algorithms of the image analysis software Image Pro 7 according to the morphology.