## **Alternative Energy and Carbon Source for Biosurfactant Production**

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Abstract : Because of their several advantages over chemical surfactants, biosurfactants have given rise to a growing interest in the past decades. Advantages such as lower toxicity, higher biodegradability, higher selectivity and applicable at extreme temperature and pH which enables them to be used in a variety of applications such as: enhanced oil recovery, environmental and pharmaceutical applications, etc. Bacillus subtilis produces a cyclic lipopeptide, called surfactin, which is one of the most powerful biosurfactants with ability to decrease surface tension of water from 72 mN/m to 27 mN/m. In addition to its biosurfactant character, surfactin exhibits interesting biological activities such as: inhibition of fibrin clot formation, lyses of erythrocytes and several bacterial spheroplasts, antiviral, anti-tumoral and antibacterial properties. Surfactin is an antibiotic substance and has been shown recently to possess anti-HIV activity. However, application of biosurfactants is limited by their high production cost. The cost can be reduced by optimizing biosurfactant production using cheap feed stock. Utilization of inexpensive substrates and unconventional carbon sources like urban or agro-industrial wastes is a promising strategy to decrease the production cost of biosurfactants. With suitable engineering optimization and microbiological modifications, these wastes can be used as substrates for large-scale production of biosurfactants. As an effort to fulfill this purpose, in this work we have tried to utilize olive oil as second carbon source and also yeast extract as second nitrogen source to investigate the effect on both biomass and biosurfactant production improvement in Bacillus subtilis cultures. Since the turbidity of the culture was affected by presence of the oil, optical density was compromised and no longer could be used as an index of growth and biomass concentration. Therefore, cell Dry Weight measurements with applying necessary tactics for removing oil drops to prevent interference with biomass weight were carried out to monitor biomass concentration during the growth of the bacterium. The surface tension and critical micelle dilutions (CMD-1, CMD-2) were considered as an indirect measurement of biosurfactant production. Distinctive and promising results were obtained in the cultures containing olive oil compared to cultures without it: more than two fold increase in biomass production (from 2 g/l to 5 g/l) and considerable reduction in surface tension, down to 40 mN/m at surprisingly early hours of culture time (only 5hr after inoculation). This early onset of biosurfactant production in this culture is specially interesting when compared to the conventional cultures at which this reduction in surface tension is not obtained until 30 hour of culture time. Reducing the production time is a very prominent result to be considered for large scale process development. Furthermore, these results can be used to develop strategies for utilization of agro-industrial wastes (such as olive oil mill residue, molasses, etc.) as cheap and easily accessible feed stocks to decrease the high costs of biosurfactant production.

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