The Effect of Electromagnetism on Growth and Flocculation Potential of some selected Yeasts in Batch and Fed Batch Cultivation Systems

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Abstract : **In this study, growth and flocculation potential of five different flocculent yeasts: *Candida sphaerica* OB10, *Kluyveromyces marxianus* OY11, *Saccharomyces cerevisiae* OGB1, *Torulopsis stellate* OGB3 and *Candida kefyr* OGB10 were investigated under electromagnetic condition in batch and fed batch cultivation systems. *Candida sphaerica* OB10 had optimum growth of 9.36 log cfu/mL under batch culture system followed by *Saccharomyces cerevisiae* OGB1 with 9.19 log cfu/mL and *Kluyveromyces marxianus* OY11 with growth of 7.72 log cfu/mL in fed-batch culture system; both without electromagnetic induction. *Torulopsis stellata* OGB3 under fed-batch system was 9.78 log cfu/mL after electromagnetic induction. Also, *Candida kefyr* OGB10 had an optimum growth of 10.14 log cfu/mL in fed-batch culture system. The results of the flocculation potential of the yeast isolates showed a reduction in flocculation potential in fed-batch culture system after electromagnetic induction as compared with fed batch system without electromagnetic induction while *Candida sphaerica* OB10, *Kluyveromyces marxianus* OY11, *Torulopsis stellate* OGB3 and *Candida kefyr* OGB10 had an increase in flocculation potential in batch culture system after electromagnetic induction. These results showed that the batch culture system which was steady and undisturbed was more important in yielding well coagulated and bottom settled cells in electromagnetically induced yeast fermentation systems when compared with the fed-batch system.**

Keywords: **Yeasts, Flocculation, Electromagnetism, Fed batch, Batch**

I. INTRODUCTION

The non-sexual cell aggregation of yeasts also known as flocculation, although dates back to Pasteur scientific notes [1], its mechanism has raised much controversy in this last century [2]. This mechanism is reported to be mediated by interactions between the receptor - α-mannan carbohydrates which bind to cell surface lectin-like proteins (flocculins) which are found on adjacent both on flocculent and non-flocculent cells. This process is reported to be inhibited by EDTA or sugars, and restored by Ca^{2+} ions with the Ca^{2+} ions acting as cofactors to maintain the active conformation of surface proteins, thereby enhancing the capacity of lectins to interact with α-mannan carbohydrates [3]. Additionally, growth and flocculation of yeasts is reportedly affected by pH of the growth medium, temperature, oxygen, sugar types, ethanol, shortages of sterols and fatty acids [4] and more importantly, genealogical and cultural age as in the case of *Saccharomyces cerevisiae* [5]. Asides these factors, cell density and mechanical agitation was also reported to affect growth and as flocculation [6]

Flocculation of yeasts has been exploited for features such as more rapid biomass recovery or clarification of fermentation products. Furthermore, flocculation is of great importance in several industrial processes as it is thought to be a spontaneous process of auto-immobilization which is an easy, cost effective, fast and eco-friendly process of cell separation from fermented broth thereby allowing for reuse (repitching), usually after washing step [7]. In addition, flocculation is reported to enhance cell concentration in the green beer for further flavour maturation and achievement of colloidal stability [6]. Apart from alcohol production, flocculation of yeasts has also found tremendous application in production of bio-ethanol [8,9]. Additionally flocculent yeasts are reported to have potential application in bioremediation of heavy metals and the production of heterogeneous protein [10,11]. It has been shown that some highly flocculent cultures lost their flocculation characteristic during the early stages of growth, in the presence of nutrients, and recovered it towards the end of the exponential growth phase coinciding with nutrient depletion, hence, flocculation is regarded as an important property in an environment with limited nutrients as autolysis and death of cells inside flocs can provide nutrient in the surrounding cells [4].

Electromagnetic fields (EMF) are reported to have a major impact on biological systems including microorganisms and microorganisms are being exposed to radiofrequency and microwaves radiation signals from several sources [12]. Because of this reported exposure, the effect of EMF on microbial growth and physiology has attracted tremendous attention recently [12,13,14]. Generally, EMF at particular frequency and power induces modulating effects resulting in an increase or decrease in the proliferation of microorganisms and these effects are no caused by any elevation in the temperature [14,15,16]. However, none of these reports investigated the effect of EMF on flocculation.

Due to the importance associated with the flocculation phenomenon observed with different type of yeasts, various methods for the enhancement of this process have been investigated. Chiefly among the methods used in improving flocculation potentials are chemical-based which not only affect flocculation but also the fermentation characteristics of the organism ultimately affecting product characteristics as in the case of beer production[17], hence the need for a more efficient method of enhancing yeasts flocculation that will not significantly alter fermentation characteristics of the organisms. In this study, we investigated the growth and flocculation potential of some yeasts under electromagnetic flux in batch and fed batch cultivation system.

II. MATERIALS AND METHODS

Microorganism and culture conditions

The yeasts used in this study are *Candida spherical* OB10, *Kluyveromycesmaxianus* OY11, *Saccharomyces cerevisiae* OGB1, *Torulopsis stellate* OGB3 and *Candida kefyr* OGB10. They were obtained from The Culture Collection of the Department of Microbiology, University of Ibadan. The organisms were sub cultured on Yeast Extract Agar (YEA) and incubated at 35 ºC for 3 days.

Screening of yeast for flocculation potential

For the screening of the flocculation ability of the yeasts, the Helm's test flocculation assay as described by D' Hautcourt and Smart, [18] was used and the flocculation potential expressed using the following formula according to Powell *et al*.[6].

$$\frac{\text{Control absorbance} - \text{Experimental absorbance}}{\text{Control absorbance}} \times 100 = \% \text{ Flocculation}$$

Growth and flocculation potential of yeasts in Batch and Fed Batch culture systems

Batch culture system

Batch culture cultivation of the yeasts was carried out in 250 ml Erlenmeyer flasks containing 50 mL of Yeast Extract Peptone Dextrose Broth (YEPD) inoculated with 1mL inoculum containing 10^8 cells. The culture was incubated for 120 hours. The growth was measured by optical density using a spectrophotometer at 600nm and converted to logarithm values [19] and flocculation potential was checked at intervals for 120 hours as previously described.

Fed batch culture system

Fed-batch experiments were done using the fermentation medium above and inoculated with 1 ml of the cell suspension and incubation carried out at ambient temperatures for 120 hours. Then, 10 ml of fresh sterile fermentation medium was added every 12 h. Each fed-batch culture was sampled periodically for cell growth and flocculation potential by aseptically removing 10 ml of the culture using a sterile syringe every 24 hrs. The growth was measured by optical density using a spectrophotometer at 600nm and converted to logarithm values [19] and flocculation potential was checked at intervals for 120 hours as previously described.

Exposure to electromagnetic field setup

Cultures to be exposed to electromagnetic filed were placed in the middle of a homogenous magnetic field generated by a solenoid consisting of 94 turns from electrically insulated 2 mm cupper wire wound in a homogenous way into a copper cylinder 2 mm thick, 10cm diameter and 5cm length as previously described Garuba *et al*. [20]. The electromagnetic field intensity calculated using the Biot-Savart law as reported by Ahmed *et al*. [21].The cultures exposed yeast isolates were placed in the middle of the coil by using supports to get a homogenous and higher magnetic field strength. The ends of the solenoid were connected to a step-down transformer fed from the mains (220 V).

$$B = \frac{2\text{-}\mu_{\circ} NIR^2}{2(R^2+\chi^2)^{3/2}}$$

Where;

B= Electromagnetic flux density (T)

I= Current of coil (A)

R= Coil Radius (m)

χ= Coil distance on axis to point (m)

N= Number of wire loops

$\mu_{\circ}$= Permeability constant

$B = 1.257 \times 10^{-6}$T.m/A

Equal volumes of the yeast isolates were exposed at their mid-to-late exponential phase (maximum rate of active growth) to the magnetic field for 15 minutes. The unexposed yeast cells served as control [22].The growth rate and flocculation potential of non-induced yeast isolate was compared to that of electromagnetically induced yeast isolate to determine if there is substantial effect of the electromagnetic inducement on the growth and flocculation potential of the yeast isolates.

III. RESULTS

The results of the growth of the yeasts in batch and fed batch cultures (exposed and unexposed to electromagnetic fields) is presented in figures 1-5. *Candida sphaerica* OB10 had the highest cell growth of 9.36cfu/ml at 72hrs under the batch uninduced cultivation system while the lowest of growth of 0.67cfu/ml at 24 hrs was observed under the batch induced cultivation system. Highest flocculation potential of 98.8% was observed at 96hrs when cells of *Candida sphaerica* OB10 were cultivated in batch under electromagnetic condition and lowest flocculation potential of 48.37% at 24hrs was observed when the batch system was not exposed to the electromagnetic condition. *Klyveromyces marxianus* OY11 had highest and lowest growth of 7.71 at 24hrs incubation and 0.68 at 24hrs in

batch and fed batch uninduced systems respectively while highest flocculation potential of 99.39% at 96hrs and lowest flocculation potential of 70.87% at 72 hrs was recorded in Batch induced and fed batch induced cultivation system. Cultivation of *Saccharomyces cerevisiae* OGB1 in batch and fed batch systems with exposure and non-exposure to electromagnetic field should highest cell growth of 9.19 cfu/mL at 72 hrs and highest flocculation potential of 93.2% at 72 hrs in batch uninduced and batch induced systems respectively, while the lowest cell growth of 0.64 cfu/ml and lowest flocculation potential of 23.9% at 72 hrs was observed in the fed batch induced system.

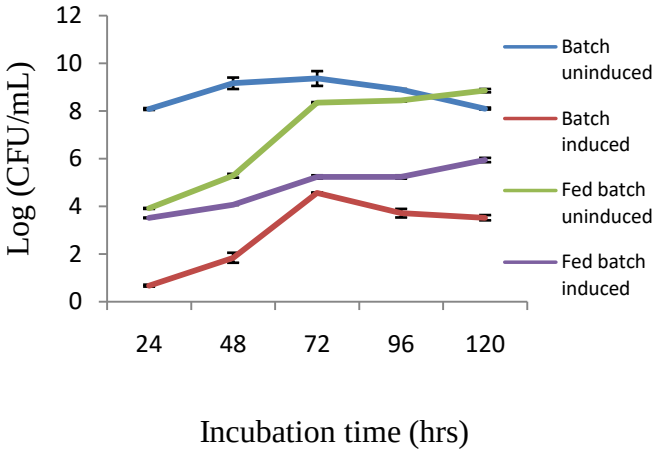

Fig 1a. Growth of *Candida sphaerica* OB10 in Batch and Fed batch systems with and without electromagnetic induction

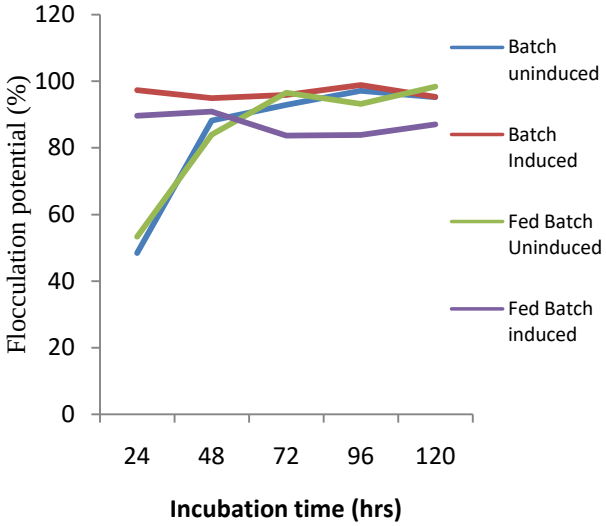

Fig1b flocculation potential of *Candida sphaerica* OB10 in Batch and Fed batch systems with and without electromagnetic induction

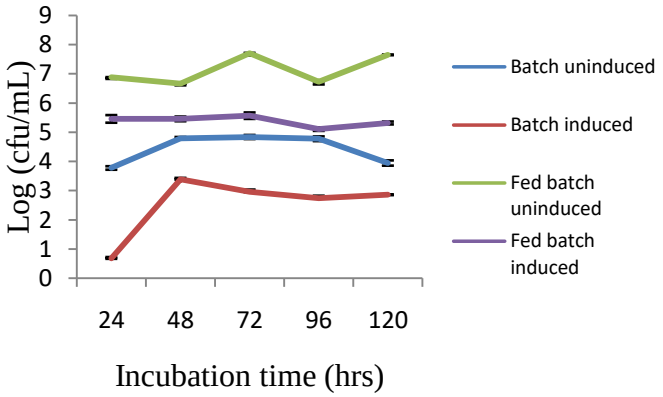

Fig 2a Growth of *Kluyveromyces marxianus* OY11 in Batch and Fed batch systems, with and without electromagnetisim

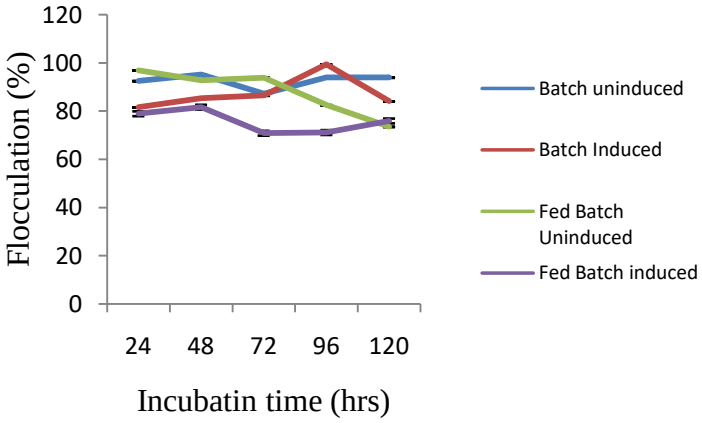

Fig 2b Flocculation potential of *Kluyveromyces marxianus* OY11 in Batch and Fed batch systems, with and without electromagnetisim

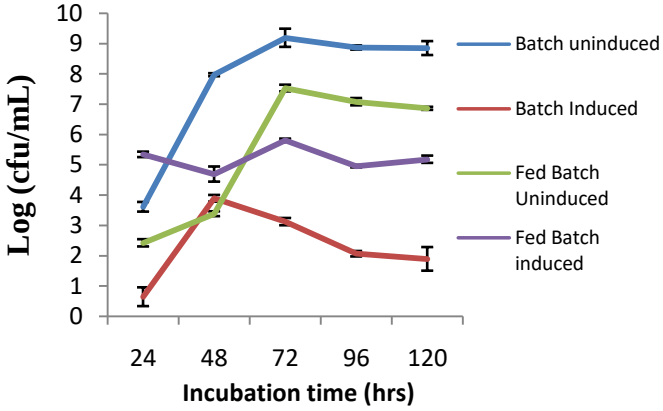

Figure 3a: Growth of *Saccharomyces cerevisiae* OGB1 in Batch and Fed batch system, with and without electromganetism

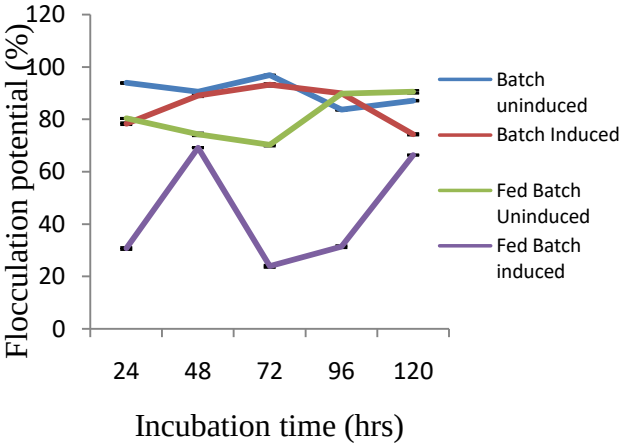

Figure 3b: flocculation potential of *Saccharomyces cerevisiae* OGB1 in Batch and Fed batch system, with and without electromganetism

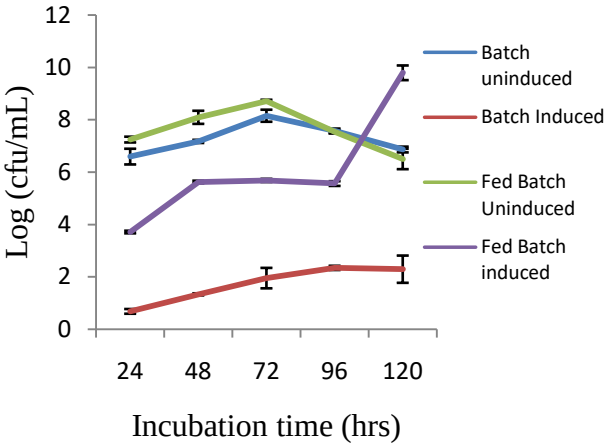

Figure 4a: Growth of *Torulopsis stellata* OGB3 in Batch and Fed batch systems, with and without electromagnetism

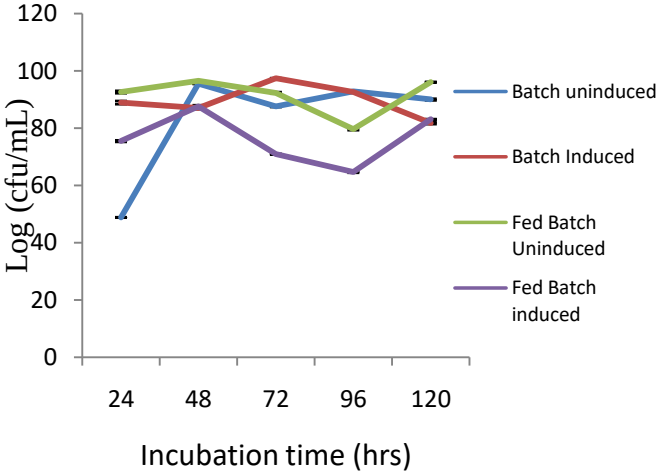

Figure 4b: Flocculation potential of *Torulopsis stellata* OGB3 in Batch and Fed batch systems, with and without electromagnetism

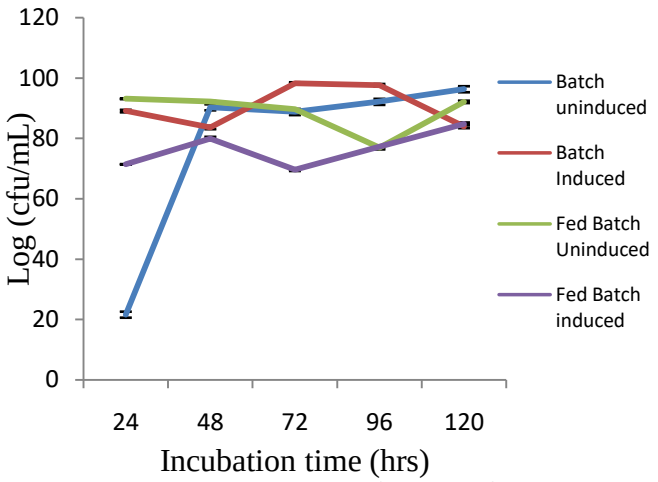

Figure 5b: Flocculation potential of *Candida kefyr* OGB10 in Batch and Fed batch systems, with and without electromagnetism

When the effect of electromagnetic induction on growth and flocculation potential by *Torulopsis stellate* OGB3 was investigated in Fed batch and Batch systems, fed batch cultivation with exposure to electromagnetic system gave the highest growth of 9.79 cfu/ml at 120hrs while the lowest growth of 0.68cfu/ml was observed in the batch system without exposure to electromagnetic system. Highest flocculation potential of 96.5% was observed in the fed batch system with non-exposure to electromagnetic field at 48hrs of incubation while the lowest flocculation potential of 48.85 was observed at 24 hrs with batch system that wasn't exposed to electromagnetic system. With *Candida kefyr* OGB10, maximum cell growth of 10.13 cfu/ml at 120hrs was observed in fed batch system without exposure to electromagnetic inducement while the lowest cell growth of 1.28 cfu/ml at 24hrs under the batch cultivation system without electromagnetic field exposure. Highest flocculation and lowest flocculation potential of 96.3 and 21.6 % at 120 and 24 hrs respectively were observed under the batch cultivation system without exposure to electromagnetic exposure.

IV. DISCUSSION

The effect of various type of biophysical factors such electric field, magnetic fields and electromagnetic fields on the functional parameters of microorganisms has received so much attention in recent years [13,14,23]. In this study, microorganisms were cultivated in batch and fed batch systems with and without exposure to low field electromagnetism and the results showed that the cultivation systems and low electromagnetic field had varying effects on the growth and flocculation potential of the yeasts investigated. *Candida sphaerica* OB10, *Saccharomyces cerevisiae* OGB1 and *Candida kefyr* OGB10 had a higher growth in batch system than the fed-batch system as observed in the study could be that nutrient addition, during fed-batch slowed down the permeability of yeast cell membrane. This in

turn could alter the growth rate of yeast; making the undisturbed batch culture more suitable for the growth of flocculent yeast as suggested by Soares [24]. On the other hand, an increase in growth was observed for *Kluyveromyces marxianus* OY11 in fed-batch culture system. Similar increase in growth in fed batch cultures has been reported by Hadiyanto *et al*., [25] who observed an increase in the growth rate of *Kluyveromyces marxianus* during Fed batch cultivation in a culture medium made of whey. This increased growth could suggest that the dynamic system of nutrient provided by the fed-batch system is more important in attaining high cell density for this organism. The growth rate of *Torulopsis stellate* OGB3 showed similar pattern in batch and fed-batch systems, showing that the growth rate of the organism is not affected by nutrient addition. A reduction in growth rate of all isolates was observed after electromagnetic induction. Sohni *et al*. [14] indicated that the reduction could be as a result of modulating effects of the inducement which could either increase or inhibit the proliferation of yeast cells. His results showed that electromagnetic inducement can induce cell death followed by their rapid recovery.

Electromagnetic inducement of flocculation in batch and fed batch systems showed that the flocculation potential of *Candida sphaerica* OB10, *Torulopsis stellate* OGB3 and *Candida kefyr* OGB10 increased in batch culture system after electromagnetic induction while a reduction in flocculation potential was observed in fed-batch culture system. This shows that electromagnetic induction as a biophysical agent might be of potential importance in yeast cells' coagulation and bottom settling in batch culture system. The increase in flocculation could be as a result of increased negativity of the yeast cell after inducement which can lead to an increased flocs formation [14]. Also the binding sites of sugar residues on yeast cells could be altered leading an increase or decrease in the ability to bind to flocculent proteins (Lectin proteins and FLO1 proteins) on other yeast cells [2,3]. A reduced flocculation potential observed in the fed-batch culture system with electromagnetic induction (as compared with the fed batch without electromagnetic induction) with some of the organisms suggest that the electromagnetic field affected the adversely altered the coagulation and bottom settling ability of the yeasts. This adverse effect could be as a result of the interaction between the electric charges generated by the EMF adversely affecting the expression or configuration of the α-mannan carbohydrates on the surface of the cell [2,3].

V. CONCLUSION

In this study, all the selected flocculent yeasts were more flocculent in batch culture system than fed-batch culture system. The batch culture system which was steady and undisturbed via periodic nutrient addition, was more important in yielding well coagulated and bottom settled cells in electromagnetically induced yeast fermentation systems when compared with the fed-batch system. Flocculation of *Torulopsis stellata*, *Kluyveromyces maxians* and *Candida sphaerica* was positively affected by electromagnetic induction leading to increased flocculation compared with the uninduced batch system. There is however the need for more investigation to elucidate this mechanism of enhanced flocculation with a view to enhance its potential use in industrial processes involving flocculent yeasts.

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AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

AUTHORS' CONTRIBUTIONS

EOG, MAA, OMA designed the work. MAA collected the data and also prepared the manuscript together with EOG. OMA and EOG analysed some of the data. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests

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