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Erythromycin production in batch and fed batch bioreactor using agro-industrial wastes.

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ABSTRACT

To reduce the cost associated with the production of erythromycin by *Saccharopolyspora erythraea* NCIMB 12462 in submerged fermentation cultures, experiments were conducted to optimize production medium composition using beet molasse, beet pulp and corn steep liquor as agroindustrial residues in shake flask level. The presence of beet molasse combined with corn steep liquor realized best erythromycin concentration. The kinetics of erythromycin production and carbohydrates consumption were studied in a laboratory 7 l stirred tank bioreactor under different stirring speeds using the previously optimized medium. The highest erythromycin concentration 231.3mg/l was attained at 600 stirring speed after 60 hours earlier than that obtained from shake flask study. When batch fermentation was carried out with lower concentration of beet molasse at the optimized stirring speed 600 rpm an increase in erythromycin production by 11.6% was observed. Supplementation of 2% corn steep liquor, 4% beet molasse and 0.2% isopropanol in the fermentation media after 48hours independently in shake flasks raised erythromycin concentration by 11.8% compared to control media without addition. Therefore, addition of 0.2% isopropanol followed by 4% beet molasse and 2%corn steep liquor in fed-batch cultivation in bioreactor increased production by 25% compared to batch cultivation without any addition.

Keywords: erythromycin, *Saccharopolyspora erythraea*, stirred tank bioreactor.

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INTRODUCTION

Erythromycin is a 14-member lactone ring belongs to macrolide group of antibiotics produced by the actinomycete *Saccharopolyspora erythraea*, formerly known as *Streptomyces erythraeus*. Erythromycin has been used clinically since 1952 and is effective to combat Gram-positive and some Gram-negative microorganisms and is applied in curing gastrointestinal, respiratory, genital tract, soft tissue and skin infections [1]. Due to its enormous importance in human health care, requirement for huge amount of erythromycin with minimum costs are needed for the continual fight against bacterial diseases. Moreover, continuous effort are being made to decrease its production cost by process optimization using agro-industrial by-products through different fermentation processes in submerged and solid state cultures [2,3]. In spite of the fact that they are highly variable in composition, agroindustrial wastes and byproducts of other industries are better substrates than others in fermentation process . The frequently utilisable sugar beet molasses contain a large amount of sugar around 33% sucrose besides various nitrogenous substances, vitamins and trace elements. Corn steep liquor (CSL) formed during starch production from corn is rich in nitrogen (about 4%) and is very efficiently utilized by microorganisms. In addition, it contains many different amino acids (alanine, valine, methionine, arginine, threonine, glutamate). Sugar beet pulp (SBP) has the potential to be a valuable substrate in various biotechnological processes. Its dry matter constitute of 60%-70% pectin , cellulose and hemicellulose. The main constituent monosaccharides in (SBP) are glucose, galacturonic acid, and arabinose (both present in pectin) [4]. Beet molasses (BM) and (CSL) were the major sources in participating to the production of cephalosporin C [5]. Whereas apple pomace was from the most suitable medium component for low-cost production of antimicrobial compounds by strain Hhs.015^T [6]. The improvement of fermentation media ingredients is an essential part in the advancement of any fermentation procedure [7]. Erythromycin production in shake flasks was attributed to the corn steep liquor and soybean flour used as a nitrogen source in fermtation media [8]. To this end, an attempt was done in our present work to optimize erythromycin production in shake flask in order to obtain the optimum fermentation medium containing agro-industrial carbon and nitrogen sources, while the information obtained is useful to large-scale fermentation using 7l laboratory scale stirred tank bioreactor for effective output of erythromycin at low cost. Earlier researches have confirmed that fed-batch strategy was looked as another possible cultivation process by many researchers to raise antibiotic production by actinomycetes like in case of rifamycins [9], daptomycin[10] and clavulanic acid production [11]. Furthermore, feeding design of carbon source was critical to erythromycin fermentation since different carbon sources like soybean oil, glucose and propanol were used altogether during erythromycin production phase, performed different roles in erythromycin biosynthesis [12].Therefore, an extended study on the effect of fed-batch fermentation with agroindustrial by-products carbon and nitrogen source on the production of erythromycin was also investigated in the bioreactor.

MATERIALS AND METHODS

Microorganism and Cultivation Conditions:

Saccharopolyspora erythraea NCIMB 12462 used in this study was obtained from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland, UK. This strain was maintained on starch nitrate agar medium of the following composition (g/l): starch, 20; $NaNO_3$, 2; K_2HPO_4, 1; $MgSO_4$. $7H_2O$, 0.5; NaCl, 0.5; $CaCO_3$, 3; $FeSO_4.7H_2O$, 0.01; agar, 20. The pH was adjusted to 7.0 before sterilization. The slants were inoculated and incubated at 32^oC for 10 days in order to obtain a heavy sporulated growth. After that time, spores were suspended in 20% (w/v) glycerol and stored in vials at –86°C. Inoculum cultures for the shake flask and bioreactor were grown by inoculating a 250-mL shake flask (50-ml working volume) with a single vial of frozen stock culture. Inoculum media containing the basal fermentation medium for erythromycin production composed of (g/l): yeast extract 1.5, starch, 3; $NaNO_3$, 3; K_2HPO_4 2, $MgSO_4 \cdot 7H_2O$ 0.5, and CaCO3, 1. The pH was adjusted to 7.2 before sterilization. Flasks were incubated at 32^oC shaking incubator operating at 200 rpm for 48 h to inoculate the production medium.

Shake flask cultivation:

Erythromycin was produced by submerged fermentation using different liquid culture media .For identifying the optimum fermentation medium, starch in the basal fermentation media was substituted by BM

provided from the Sugar and Integrated Chemicals company (El Hawamdya, Giza, Egypt) and BP a secondary by- product of sugar beet from sugar industry and glucose at 30g/l as organic carbon sources whereas $NaNO_3$ was substituted by CSL (by-product of corn wet-milling obtained from the Egyptian Starch-Glucose Factory, Mostorod) as organic nitrogen source at 4ml/l besides $(NH_4)_2SO_4$ at 2g/l as inorganic nitrogen source . In these trials nutrients as BM syrup , crushed BP and glucose were used independently and alternatively with CSL and $(NH_4)_2SO_4$ in the basal fermentation media as showed in Table 1. Shake flasks were placed on a rotary incubator shaker at 200 rpm and 32°C for 144 h. All the fermentations were carried out in triplicate. After that time, the media were filtered and analyzed for the determination of final pH and erythromycin concentration.

Table 1: Media composition screened for the production of erythromycin

Production media	Composition (g/l)
Basal fermentation media	yeast extract, 1.5; starch, 3; $NaNO_3$, 3; K_2HPO_4, 2; $MgSO_4 \cdot 7H_2O$,0.5 ; $CaCO_3$, 1
M1	yeast extract, 1.5; glucose 30; $NaNO_3$, 3; K_2HPO_4, 2; $MgSO_4 \cdot 7H_2O$,0.5 ; $CaCO_3$, 1
M2	yeast extract, 1.5; BM, 30; $NaNO_3$, 3; K_2HPO_4, 2; $MgSO_4 \cdot 7H_2O$,0.5 ; $CaCO_3$, 1
M3	yeast extract, 1.5; BP, 30; $NaNO_3$, 3; K_2HPO_4, 2; $MgSO_4 \cdot 7H_2O$,0.5 ;$CaCO_3$, 1
M4	yeast extract, 1.5; BM, 30 ; CSL,4ml ; K_2HPO_4, 2; $MgSO_4 \cdot 7H_2O$,0.5 ; $CaCO_3$, 1
M5	yeast extract, 1.5; BP, 30; CSL,4ml; K_2HPO_4, 2; $MgSO_4 \cdot 7H_2O$,0.5 ; $CaCO_3$, 1
M6	yeast extract, 1.5; BM, 30; $(NH_4)_2SO_4$, 2; K_2HPO_4, 2; $MgSO_4 \cdot 7H_2O$,0.5 ; $CaCO_3$, 1
M7	yeast extract, 1.5; BP, 30; $(NH_4)_2SO_4$, 2; K_2HPO_4, 2; $MgSO_4 \cdot 7H_2O$,0.5 ; $CaCO_3$, 1

Batch bioreactor fermentation:

Batch fermentations was performed in a 7-l stirred-tank bioreactor (bioflow 310; New Brunswick Scientific, Edison, NJ, U.S.A.) containing the previously optimized production medium obtained from shake flask cultivation at pH 7.The useful volume used in the bioreactor was 5-l. The bioreactor is equipped with digitally controlled pH electrode, temperature probe, polographic DO electrode (Ingold, Mittler-Toledo ,Switzerland) and two six-blade Rushton turbine impellers (5.2 cm diameter), fixed on the agitator shaft above 3.2 cm air sparger. The pH electrode was calibrated by using standard buffers (Fluka) at pH 7 and 9 prior to the sterilization of fermentor (121°C for 20 min). However, the calibration of DO electrode was conducted after sterilization by the air until 100 % saturation was achieved. The foam was controlled manually by adding few drops of silicon based antifoam (Sigma) at the time of foaming . The temperature in the 7-l vessel was controlled at 32°C. The airflow rate was set at (1 v/v/m) using filtered sterile air. For the comparison between different stirring effect on the production without pH control, different stirring speeds (200, 400 and 600 rpm) by varying the stirring speed rate in the bioreactor were allowed. The suitability of the stirring speed was determined on the basis of the results obtained. The cultivation comprised the transferring of 10% (v/v) of vegetative culture 48h growth directly to the bioreactor.

Morphological features:

A semi-automatic image analysis method was used to characterize the morphology of *S. erythraea* in samples taken from the STR at the same time each 48h. Olympus CX41 optical microscope (model LC20, GMBH, Münster, Germany) with digital camera output connected to a computer monitor was used to take photos from slides prepared from a stained drop sample by lactophenol blue.

Effect of different addition in shake flasks on erythromycin production:

Three different components were added at different concentrations were added separetly after 48h incubation time in shake flask on the same optimized medium. Among the feeds tested, addition of CSL with concentration of 0.5%, 1%, 2% and 4%, BM with 1%, 2% and 4% besides another set of cultures was supplemented with isopropanol 0.2%, 0.4%, 0.6% and 0.8%. All feeding experiments were compared with control cultures without addition. Based on addition optimization results, the best feeds were applied on the aforementioned stirred tank bioreactor during fermentation time. Erythromycin titers have been measured throughout the whole process.

Fed-batch bioreactor fermentation:

The cultivation comprised two phases: (1) initial cultivation in the production media under the optimized stirring speed resulting from the bioreactor batch fermentation and (2) a fed-batch cultivation with external feeding (a feed solution contained the preferable nutrient concentration based on results obtained previously from shake flask fed-addition cultivations).

Determination of erythromycin:

Samples were taken at different times during cultivation in falcon tubes for centrifugation at 3,000 rpm for 15 min. Supernatant was used for pH, carbohydrates and erythromycin determination. The amount of the erythromycin produced in the fermentation broth was determined by means of biological method [13]. The antibiotic assay medium (Difco) composed of (g/l): glucose, 10.0; peptone, 10.0; meat extract, 2.5; yeast extract, 5.0; NaCl, 10.0 and agar, 20.0. The erythromycin sensitive strain *Bacillus subtilis* NRRL B-543 was used for biological activity determination. The diameters of the inhibition zones obtained were measured and the amount of erythromycin produced was calculated from a biological standard curve previously made using extra pure standard erythromycin (Sigma, USA) as an authentic reference material.

Estimation of total carbohydrates:

The total carbohydrates were determined spectrophotometrically [14]. The samples were heated with sulfuric acid to hydrolyze the polysaccharides and hydrate the monosaccharides to form furfural from pentoses and hydroxymethylfurfural from hexoses. The solutions of furfural and hydroxyfurfural were then treated with a phenol reagent to produce a colored compound, and measured spectrophotometrically using Agilent technologies Cary 100 series UV-Vis spectrophotometer

RESULTS AND DISCUSSION

Effect of fermentation medium:

Optimization of production media components using agro-industrial by-products was studied. The results in shake-flask experiments revealed that, combination between BM and CSL medium 4 (M4) gave the highest concentration of erythromycin about 231.8mg/l compared to other media under study (Fig.1). All final pH values after 144h of incubation time were ranged from 8.3-8.6 (data not shown). It was previously reported that supplementing CSL in the medium was useful for erythromycin production and the output exeeded that of the control [15]. These observations are consistent with other findings which indicated that the addition of sugar cane molasses (a sole carbon source) at a concentration of 60 g/l accompanied by CSL (as organic N-source) in association with ammonium sulphate (as inorganic N-source) allowed the maximal erythromycin production [2]. Therefore, in our case (M4) was the most suitable medium for *Saccharopolyspora erythraea* to produce erythromycin among the seven fermentation media examined.

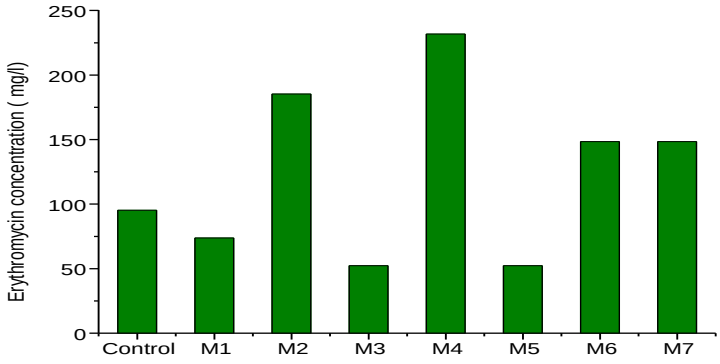

Figure 1: Effects of fermentation media on erythromycin production.

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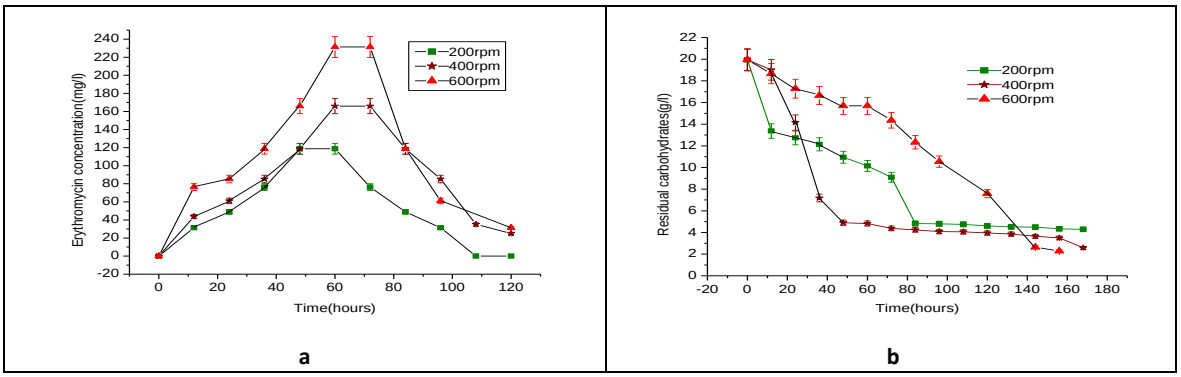
Batch cultivation of *Saccharopolyspora erythraea* in stirred tank bioreactor:

With the aim of up-scaling the shake flask process, batch cultivation was carried out with different stirring speed 200, 400 and 600 rpm in the 7-l stirred tank bioreactor using the previously optimized production medium M4 . This vessel was chosen based on the ease of measurements, close control over process parameters and comparatively good mixing leading to increased homogeneity. The relation between antibiotic production and substrate consumption as a function of different stirring speeds was calculated. In culture under stirring speed 200 rpm, an erythromycin production rate [Qp] of about 2.474 mg/l/h was calculated during the first 54h of cultivation after which a degradation rate of 1.982 mg/l/h until the end of the fermentation period was recorded. However, a longer production phase of erythromycin in 400 rpm reaching its highest value at 60h with a production rate of 2.304 mg/l/h was observed, after this time a decrease in erythromycin production until approximately 132h by a degradation rate of 2.77 mg/l/h was detected. The highest stirring speed 600 rpm recorded the highest erythromycin amount attained [Pmax] 231.3 mg/l at about 60h (Fig.2a) with a production rate of 3.21mg/l/h calculated from the beginning of fermentation period higher than that calculated from stirring speed 200 and 400 rpm. A degradation in this rate with time by 4.2 mg/l/h until the end of the fermentation time was noticed. Similar observations with cyclosporin A produced by *Tolypocladium inflatum* cultured in STR. It was observed that a considerable reduction in the antibiotic concentration after its production phase [16]. It was observed that the yield coefficient Y p/s (erythromycin produced (g/l) / mass of carbohydrates consumed (g/l)) recorded the highest value of 4.260 g/l in case of stirring speed 400 rpm at 96h followed by 1.046 g/l at the same incubation time and 0.21g/l at 48h in case of stirring speed 200 and 600 rpm respectively. These results indicated that in batch cultivation at 400 rpm the produced cells were more active towards erythromycin production compared to cultures under 200 and 600 rpm despite the fact that erythromycin concentration was the highest in case of 600 rpm under the same cultivation conditions. It has been reported that the agitation speed affected antibiotic production; the greatest actinomycin-D production occurred with aeration of 1.5 v/v/m and a stirring speed of 500 rpm [17]. Also high lovastatin titers were attained in oxygen-enriched fermentations at 300 and 600 rpm [18]. In all batch cultures, carbohydrates were consumed gradually until the end of fermentation time but no depletion was occurred (Fig.2b). In 200 stirring speed, consumption rate of carbohydrates [Qs] was (0.142 g/l/h) more than 0.1 and 0.06 g/l/h calculated from stirring speed 400 and 600 rpm respectively. It was also noticed that the pH of all cultures increased and tend to reach alkaline values similar to shake flask culture gradually after inoculation parallel to erythromycin production and reached its highest value of 8.45 at 200 rpm and 8.9 in case of stirring speed 400 and 600 rpm (Fig.2d).Generally, it was observed that in spite of erythromycin concentration degradation during fermentation time, pH stay consistent at its elevated value until the end of fermentation time.

The data also revealed differences between the depletion in the dissolved oxygen which was significant at 200 rpm. During the course of fermentation a gradual decline of the DO level was observed from 100% to 14% when 120 h was attained whereas a reduction to 16% and 32.6% at 400 and 600 rpm stirring speed at 120h and 96h respectively was noticed. In all fermentation cultures under study, during the growth phase, the DO decreased and reached a minimal value and then increased gradually thereafter as a function of the cell-growth termination when the residual carbohydrates decreased (Fig.2c). From these results, the erythromycin concentration at stirring speed 600 rpm was the highest and was chosen as the optimal one for further experiments in STR.



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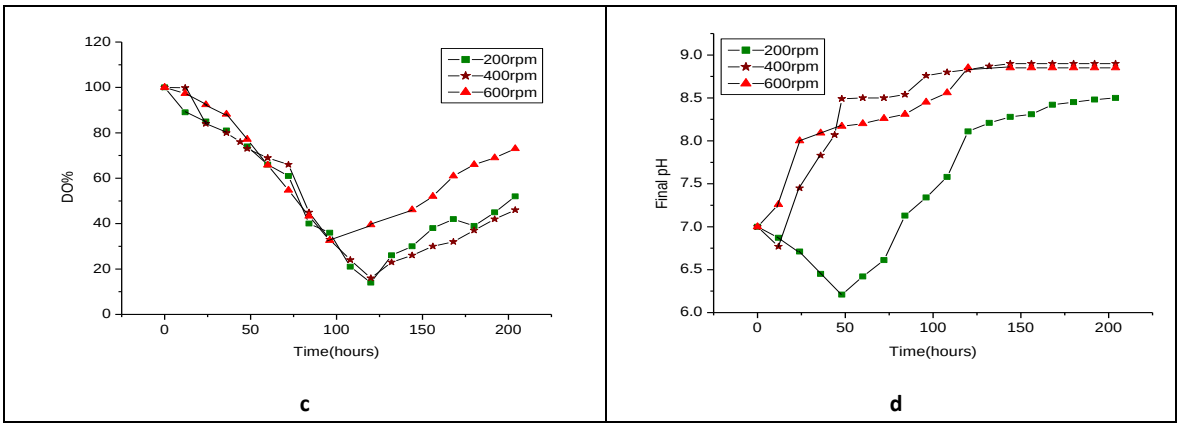

Figure 2: Time-course profile of a) erythromycin production b) residual carbohydrates c) dissolved oxygen and d) final pH during batch cultivation of *Saccharopolyspora erythraea* in 7l stirred tank bioreactor under different stirring speed.

Investigation of batch fermentation at 600 stirring speed with lower beet molasse concentration:

It was reported previously that the decrease in growth rate, addition of an inducer or consumption of a nutrient motivate the synthesis of antibiotics [19]. From this fact, an investigation was carried out aimed to raise erythromycin concentration during fermentation time on the onset of carbohydrates depletion. Reduction of BM concentration in production media from 30g/l to 10g/l resulted in an increase of the production by 11.6%. To better illustrate the results of residual carbohydrates, erythromycin production, pH and DO% during the batch fermentation, all were plotted together in Fig.3.

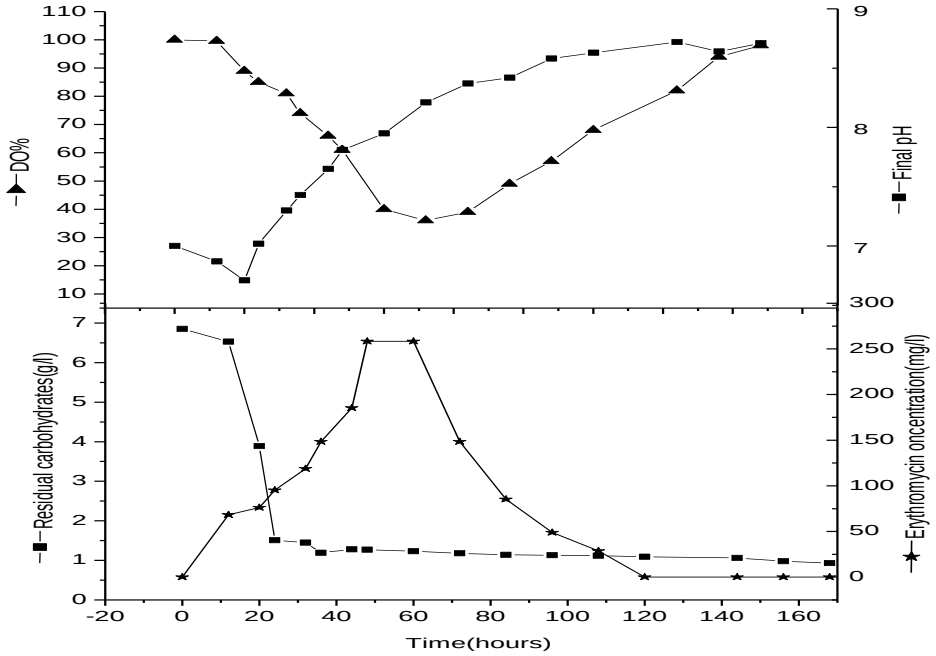

Figure 3: Time-course profile of erythromycin production; residual carbohydrates; pH and dissolved oxygen during batch cultivation of *Saccharopolyspora erythraea* in 7-l stirred tank bioreactor under 600 rpm stirring speed at lower BM concentration (10 g/l)

Erythromycin amount increased up to approximately 60 hours of processing time, with a production rate of 5.6 mg/l/h and then decreasing with time by a degradation rate of 5.38 mg/l/h. Consumption of carbohydrates recorded a rate of 0.171 g/l/h from the beginning of fermentation time until complete depletion at 32h before the raise of erythromycin concentration. On the other hand, dissolved oxygen (DO)

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level falls earlier by 24h than in batch fermentation with higher concentration of (BM) at the same 600 stirring speed. It was seen that elevation of pH from the beginning of fermentation times almost the same in all previously conventional batches. Ordinarily the higher cell mass created through utilization of higher supplements is required to bring about higher amount of any fermentation product. It has been reported that the demand of high dissolved oxygen in submerged fermentation of polyketide compounds natamycin, lovastatin and erythromycin to attain high productivity with lower cell mass supports our detections in case of erythromycin production with lower beet molasse concentration [20]. It was found that the production of gibberellic acid improves with the assumption of avoiding the increase in the biomass during the production phase [21]. To clarify the behaviour of *Saccharopolyspora erythraea* toward erythromycin production and carbohydrates consumption in all batch cultivations in STR, kinetic parameters were represented in Table 2.

Table 2: Kinetic parameters for erythromycin production and carbohydrates consumption during *Saccharopolyspora erythraea* batch cultivations in 7-l stirred tank bioreactor under different stirring speed in cultivation with 30g/l BM and under 600 stirring speed in cultivation with 10g/l BM

Kinetic Parameters	Stirring speed with 30g/l BM			Stirring speed with 10g/l (BM)
	200	400	600	600
$P_{max\text{-}vol}$ (mg/l)	118.8	165.9	231.3	258.3
$P_{max\text{-}time}$ (h)	60	72	72	60
Q_p (mg/l/h)	2.47	2.30	3.21	5.6
Q_s(g/l/h)	0.142	0.1	0.06	0.049
$Y_{p/s}$(g/gcarbohydrates)	10.46	42.6	2.1	185.3

$P_{max\text{-}vol}$ maximal volumetric erythromycin production ; $P_{max\text{-}time}$, time of maximal erythromycin production; Q_p, erythromycin production rate; Q_s ,carbohydrates consumption rate;$Y_{p/s}$, coefficient production concentration over substrate.

Growth morphology:

The effect of various ameters such as shear, biomass concentration and medium components on the morphology of this actinomycete has been previously reported [22]. The features of clumps, semi dispersed and loose mycelia are shown in Fig.4. These images were collected at 100x magnifications to highlight the differences between the three forms in different times early, middle and late time of batch fermentation.

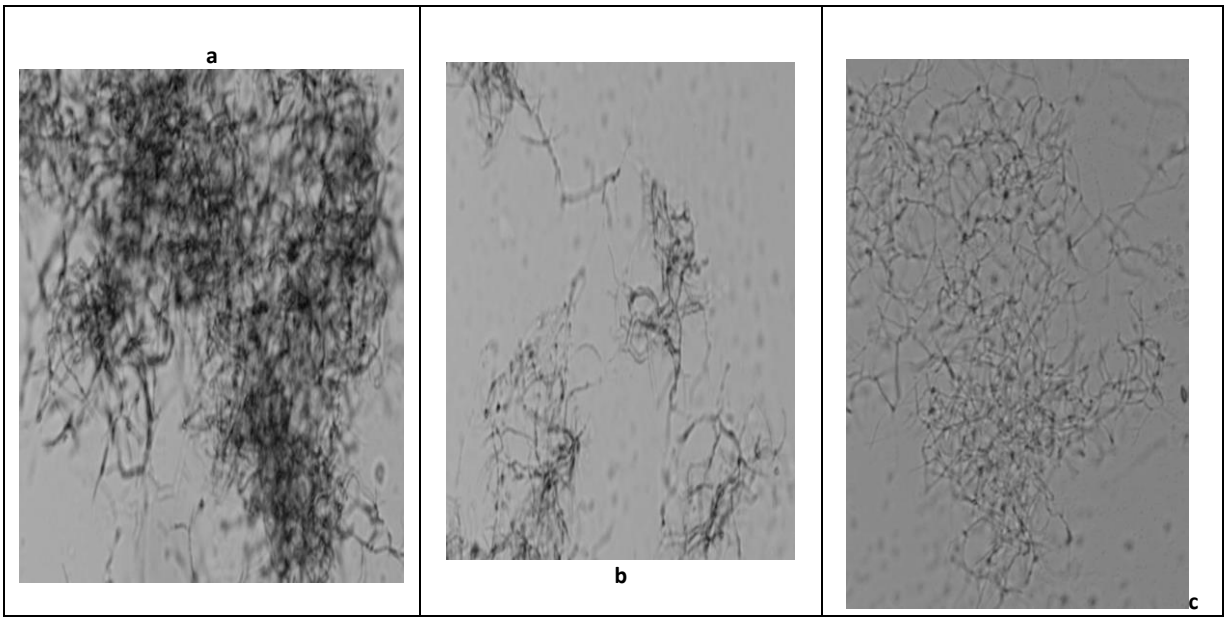

Figure 4: Microscopic observation of *Saccharopolyspora erythraea* cells at early fermentation time showing mycelia clump, b: dispersed mycelia at middle fermentation time and c: freely dispersed mycelia and spores at late fermentation time of batch cultivations in 7-l stirred tank bioreactor.

In order to seek the best feeding strategy of agroindustrial byproduct in *Saccharopolyspora erythraea* fermentation, the effect of isopropanol, CSL and BM in different concentrations on erythromycin production were tested. During feed addition in shake flasks, erythromycin level were increased by addition of 0.2% isopropanol, 1% CSL and 4% BM independently at 48hours of fermentation time. This early time of fermentation attributed to the fact that the enzyme system controlling the step of starting biosynthetic pathway of the antibiotic is more active during the exponential growth phase [23]. It was observed that an increase of about 11.85% in production exeeded that of control media without any supplementation was noticed as showed in Fig.5. Similarly, the addition of propanol is an effective strategy to increase natamycin yield [24]. Based on these results, the addition of isopropanol, CSL and BM to the fermentation medium in STR at their optimized concentration was chosen as the best nutrients for production of erythromycin in the following fed-batch experiment.

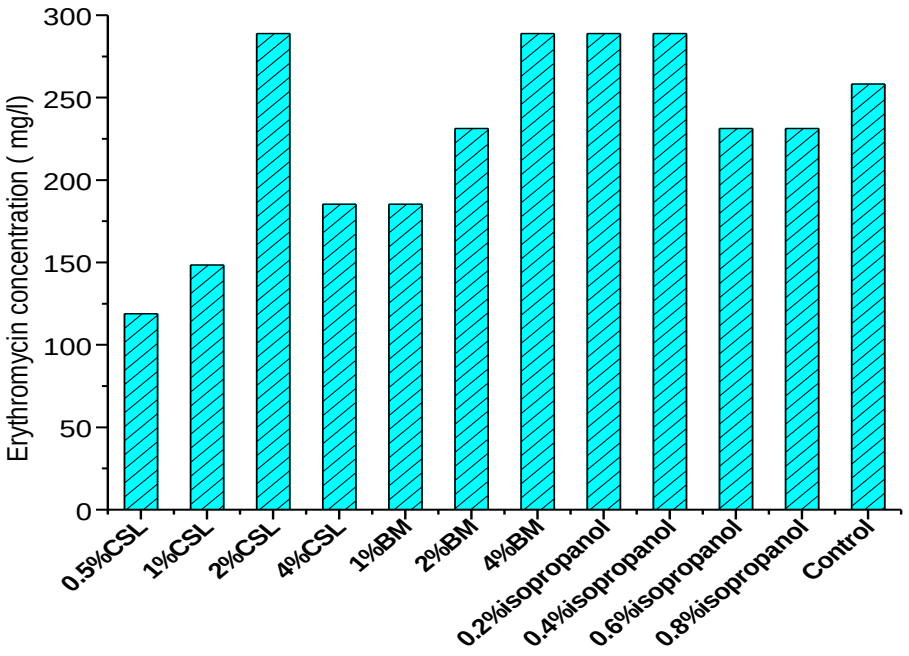

Figure 5: Effect of different addition on the production of erythromycin in shake flask.

Investigation of feed addition in STR:

By analyzing spent media in the batch cultivation in STR at lower BM concentration, it was found that erythromycin production was decreased after 60h. To minimize this reduction, 0.2% isopropanol was fed near this time to the culture followed by 1% CSL and 4% BM as well after two days from the first fed. The addition of these nutrients delayed the decrease in erythromycin production and prolonged its raise for 48h compared to previously batch cultivation without feeding. It was found that erythromycin concentration increased linearly by a production rate of 3.837mg/l/h until the fermentation time reached approximately 60 hours before the first fed by 0.2% isopropanol after which a production rate of 2.43 mg/l/h was attained untill reached 96h. After that time, second fed was carried out by addition of 1% CSL and 4% BM together at 108h incubation time. Beginning with this time, an increase in concentration of erythromycin reached 322.9 mg/l with a production rate of 5.765 mg/l was detected after which a steady value of erythromycin amount was produced followed by a significant decline of production until the end of fermentation time. Consumption of carbohydrates recorded a rate of 0.09 g/l/h from the beginning of fermentation time until the first addition after which an increase in residual carbohydrates was observed. Then a consumption rate of 0.018 g/l/h was calculated till the time of second addition followed by a consumption rate of 0.0236 g/l/h until total depletion of carbohydrates parallel to the decline of erythromycin concentration. On the other hand, dissolved oxygen (DO) level falls weakly until reached its minimum value at 48h and began to rise after the first addition similar

trend was appeared following the second addition as indicated in Fig.6. It was seen that elevation of pH towards alkalin values from the beginning of fermentation time almost the same in all previously conventional batch fermentation in STR.

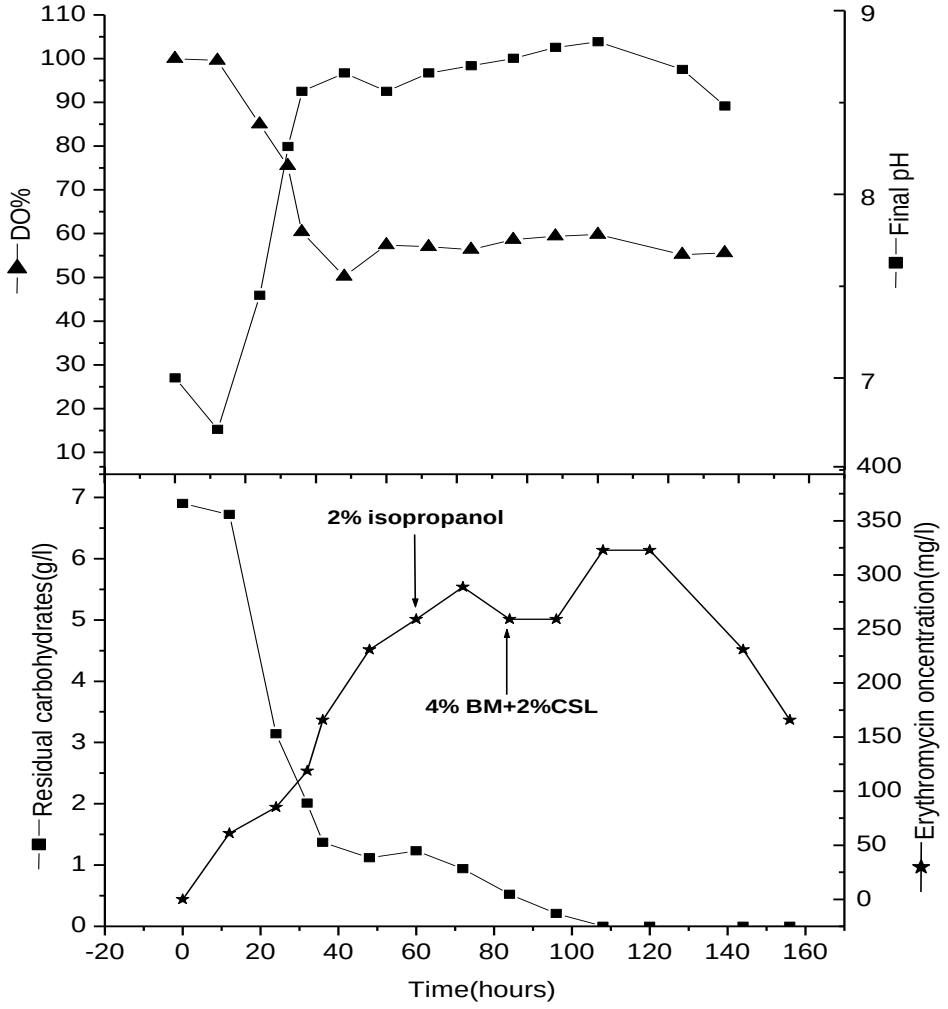

Figure 6: Time-course profile of erythromycin production *by Saccharopolyspora erythraea* showing residual carbohydrates; pH and dissolved oxygen during fed-batch cultivation with in 7-l stirred tank bioreactor under 600 stirring speed

CONCLUSION

From the obtained results, we conclude that the optimized cultivation medium containing agroindustrial byproducts beet molasse (BM) and corn steep liquor (CSL) is useful for efficient erythromycin production in submerged culture by *Saccharopolyspora erythraea* NCIMB 12462 in shake flask level . The extended investigation of the up-scaling of the fermentation process in 7l laboratory scale stirred tank bioreactor revealed that the highest production was obtained from batch cultivation at 600 stirring speed in comparaison with other lower stirring speed using the optimized low cost medium . The attempt of lowering BM concentration in order to acceleration cell growth late phase in batch fermentation at the optimized stirring speed showed a higher production than previous batches using higher BM concentration. Also the effect of the addition of 0.2% isopropanol, 1% CSL and 4% BM in shake flasks gave higher production than cultures without any supplementation. Application of these results by adding 0.2% isopropanol followed by1% CSL mixed with 4% BM in scale up fed-batch process resulted in a further increase reached its highest value at 120h and remain at a fixed concentration for 24h than a decline in production was observed. This nutritional modification of fed addition will encourage the scaling up into factory size fermenters.

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REFERENCES

[1] Dubravko J, Roberto A. Antibiot. 2016; 5: 29.
[2] El-Enshasy HA, Mohamed NA, Farid MA, El-Diwany AI. Bioresour. Technol. 2008; 99: 4263–4268.
[3] Noor El Deen AM, Abdelwahed NAM, Shata HMA, Farid MAM. J Pure Appl Microbiol. 2015; **9**: 41-48.
[4] Leijdekkers, AG, Bink JP, Geutjes S, Schols HA, Gruppen H. Bioresour Technol. 2013**;** 128: 518–525.
[5] Lotfy WA. Bioresour. Technol. 2007; 98: 3491–3498.
[6] Xinxuan W, Lili H, Zhensheng K, Heinrich B, Xiaoning G. J Biomed Biotechnol. 2010; 2010: 10.
[7] Poddar A, Gachhui R, Jana SC. J Biochem Technol. 2012 ; 3: 370-374.
[8] Sidkey NM, Abo-Shadi MAAR, Al-Mutrafy AM, Sefergy F, Al-Reheily N. J Am Sci. 2010; 6 :926-939.
[9] El Enshasy HA, Beshay UI, El-Diwany AI, Omar HM, El-Kholy AE, El-Najar R. Acta Microbiol Polonica. 2003; 51: 301-313.
[10] Ng IS, Ye C, Zhang Z, Lu Y, Jing K. Bioprocess Biosyst Eng. 2014; 37: 415-423.
[11] Saudagar PS, Singhal RS. Bioresour Technol. 2007; 98: 2010-2017.
[12] Chen Y, Wang Z, Chu J, Xi B, Zhuang Y. Bioprocess Biosyst Eng. 2015; 38(1):105–112.
[13] Bershtein EM, Vasilyeva NG, Polyak MS. Antibiotiki. 1983; 28: 250-254.
[14] Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F. Anal Chem. 1956; 28: 350-356.
[15] Xiang Z, Hai-feng H, Ju C,Ying-ping Z, Si-liang Z. Bioresour Technol. 2009; 100:1406–1414.
[16] El-Enshasy H , Abdel-Fattah Y, Atta A, Anwar M, Omar H, Abou El Magd S, Abou Zahra R. J Microbiol Biotechnol 2008;18: 128-134.
[17] Sousa MFVQ, Lopes CE, Pereira Jr N. Braz. J Chem Eng. 2002; 19: 277-285.
[18] Caas LJL, Sánchez PJA, Fernández SJM, Rodríguez PEM, Chisti Y. J Biotechnol. 2005; 16: 61–77
[19] Sánchez S, Chávez A, Forero A, García-Huante Y, Romero A, Sánchez M, Rocha D, Sánchez B, Avalos M, Guzmán-Trampe S, Rodríguez-Sanoja R, Langley E, Ruiz B. J Antibiot. 2010 ; 63: 442-59.
[20] Lale GJ, Gadre RV. AMB Expr. 2016; 6:34.
[21] Astrana LM., Gonzalez MP, Torrado A, Murado MA. Biotechnol Lett.1995; 17: 263-268.
[22] Rostamza M, Noohi A, Hamedi J. DARU. 2008;16: 13-17.
[23] Elsayed EA, Farid MA, El-Enshasy HA. Proc Biochem. 2013; 48:183-1838.
[24] Li M, Chen S, Li J, Ji Z. Appl Biochem Biotechnol. 2014; 172: 3424-3432.