**BT307 Biochemical Engineering**
**Choose-Focus-Analyze Report**

# Modeling Bacterial Growth in Human Eye During Conjunctivitis

*Analysis by -*
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# 1. ABSTRACT

Conjunctivitis is one of the most common and treatable eye infections in children and adults. Often called "pink eye," it is an inflammation of the conjunctiva, the tissue that lines the inside of the eyelid. One of the most common organisms responsible for this infection is *Staphylococcus aureus.* In this analysis, the human eye (specifically that of Abhishek Tiwari) is modeled as a Fed-batch reactor during the infection. The growth of *Staphylococcus aureus* is modeled as a function of time. Finally, the time required for eradication of the pathogen from the conjunctival tissues, under the influence of natural healing and healing aided by treatment with the antibiotic, moxifloxacin, is projected and compared.

# 2. INTRODUCTION

The mucous membrane that lines the inner surface of the eyelid and the exposed surface of the eyeball is called conjunctiva. The inflammation of conjunctiva, accompanied by redness, swelling and watery discharge is known as conjunctivitis. This condition can be attributed to one of the following reasons:

1) Bacterial infection
2) Viral infection
3) Allergic reaction.

Bacterial conjunctivitis, which is usually a benign self-limited illness, sometimes can be serious or signify a severe underlying systemic disease. The surface tissues of the eye and the ocular adnexa are colonized by normal flora such as streptococci, staphylococci, and *Corynebacterium* strains **[1]**. The organic molecules in the tears act as carbon source for the bacteria. Alterations in the host defense or in the species of bacteria can lead to clinical infection. An alteration in the flora can occur by external contamination, by spread from adjacent sites, or via a blood-borne pathway. The most common pathogenic organisms in adults include *Haemophilus* species, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and, increasingly, Gram negative rods—for example, in contact lens wearers **[2]**.

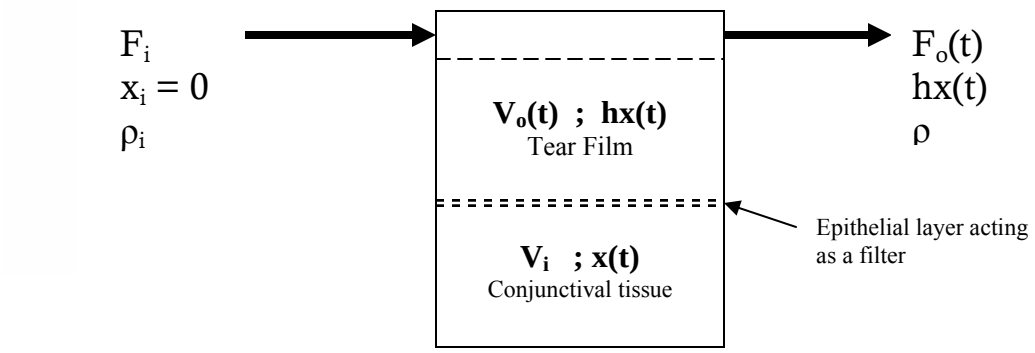
The eye has a battery of defenses to prevent bacterial invasion. The primary defense against infection is the epithelial layer covering the conjunctiva. Other defenses include bacteriostatic lysozymes and immunoglobulins in the tear film, the shearing force of the blink, the immune system in general, and non-pathogenic bacteria that colonize the eye and compete against external organisms that try to enter. When any of these defense mechanisms break down, pathogenic bacterial infection is possible **[3]**.

Invading bacteria, and the exotoxins they produce, are considered foreign antigens. This induces an antigen-antibody immune reaction and subsequently causes inflammation. In a normal, healthy person the eye will fight to return to homeostasis, and the bacteria will eventually be eradicated.

*Staphylococcus aureus* is Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes. *S. aureus* colonizes mainly the nasal passages, but it may be found regularly in most other anatomical locales. It is a facultative anaerobe that grows by aerobic respiration or by fermentation that yields principally lactic acid. *S. aureus* can grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 15 percent. *S. aureus* produces and secretes many proteins, including coagulase, protein A, alpha-, beta-, gamma-, and delta-toxin, and leukocidin, all of which could contribute to the virulence of the organism. Alpha-toxin is a pore-forming hemolytic toxin that causes membrane damage to many types of mammalian cells. The cytolytic nature of alpha-toxin for several cell types could be an important mechanism for conjunctivalal epithelial and stromal tissue damage during *S. aureus* conjunctivitis **[4]**.

# 3. MODELS

The eye can be modeled as a fed-batch reactor due to time dependence of the tear drop formation and fall. The main region of bacterial growth is the conjunctival tissue. The growth in tear film is neglected due to the blinking action of the eye and high concentrations of lysozymes. The epithelial layer of the conjunctiva acts as a filter and prevents the flow of bacteria from the tear film to the conjuntival tissue and vice versa. Thus the concentration of cells can be maintained differently in the tissue and in the tears. The organic molecules in the tear film and the tissue cells act as the source of carbon. This source can be assumed to be continuously replenished and hence the substrate concentration is assumed very high and the growth rate of *S. aureus* can be assumed constant (maximum).




*Cell Balance*:

$$\dot{I} + \dot{G} - \dot{C} - \dot{O} = \frac{dA}{dt}$$

$$\Rightarrow 0 + \mu_g x V_i - F_o hx - \mu_d x V_i = \frac{d(xV)}{dt} + \frac{d(hxV_o)}{dt}$$

$$\Rightarrow (\mu_g - \mu_d) x V_i - F_o hx = (V_i + hV_o)\frac{dx}{dt} \qquad \ldots(i)$$

*Total Mass Balance:*

$$\dot{I} + \dot{G} - \dot{C} - \dot{O} = \frac{dA}{dt}$$

$$\Rightarrow \rho_i F_i + 0 - F_o \rho - 0 = \frac{d[\rho(V_i + V_o)]}{dt} \qquad \text{Assuming } \rho_i \approx \rho$$

$$\Rightarrow F_i - F_o = \frac{dV_o}{dt} \qquad \ldots (ii)$$

Substituting back in (i),

$$(\mu_g - \mu_d)xV_i - F_o hx = (V_i + hV_o)\frac{dx}{dt} + hxF_i - hxF_o$$

$$\Rightarrow \frac{(\mu_g - \mu_d)V_i - hF_i}{V_i + hV_o} = \frac{1}{x}\frac{dx}{dt} \qquad \ldots \text{(iii)}$$

The whole period of bacterial growth can be divided into 2 phases:

**PHASE I:**

The *S. aureus* cells multiply without inducing the immune response by the body. Presence of lysozymes, immunoglobulins in tear film and some other factors are a major reason for death of *S. aureus* in this phase. These phenomenon do not change with time and the death rate of *S. aureus* can be considered a constant over phase I. The rate of formation of tear is very low and can be neglected in this phase. Thus, the volume of the reactor (tear film volume) can be assumed to be constant. Hence, in phase I, the equation (iii) takes the following form:

$$\frac{(\mu_g - \mu_d)V_i - hF_i}{V_i + hV_o} = \frac{1}{x}\frac{dx}{dt} \qquad \mu_d = k_{dl} \text{ (constant)}, F_i \approx 0$$

$$\Rightarrow \frac{(\mu_g - k_{dl})V_i}{V_i + hV'}x = \frac{dx}{dt} \qquad V' = \text{minimum volume of tear film}$$

$$\Rightarrow \int_{t=0}^{t} \frac{(\mu_g - k_{dl})V_i}{V_i + hV'} = \int_{x=x_0}^{x} \frac{dx}{x}$$

$$\Rightarrow \frac{(\mu_g - k_{dl})V_i}{V_i + hV'}t = \ln\left(\frac{x}{x_o}\right) \qquad \ldots \text{(iv)}$$

**PHASE II:**

During the phase II, *S. aureus* keeps on dividing without inducing any significant immune response. It also produces several exotoxins, such as α-, β-, γ-, δ-toxin during this period. Out of these toxins, α-toxin is the major virulence factor of *S. aureus* **[5]** (Assuming the results in **[5]** for infection of cornea are approximately valid for infection of conjunctiva also). Thus, it can be assumed that the lysis of

the cells of conjunctival tissues can be solely attributed to α-toxin. The lysis of tissue cells induces an immune response in the body. This is accompanied by an increased blood and tear flow in the affected region (causing redness) along with swelling and a slight decrease in pH of the tears and a slight increase in temperature **[6]**. The concentration of phagocytotic molecules, like neutrophils, also increase tremendously and, after a certain time, starts leveling off to become constant, till all the cells of the pathogen are eradicated from the system. The growth of neutrophils is assumed to follow the logistic equation. The specific death rate of *S. aureus* is assumed to be directly proportional to the number of neutrophils present in the system. Also, the death rate due to changes in pH and temperature are neglected and the death rate due to lysozymes etc. is considered same as that in phase I. Thus, for phase II, the equation (iii) can be written as:

$$\frac{(\mu_g - \mu_d)V_i - hF_i}{V_i' + hV_o} = \frac{1}{x}\frac{dx}{dt}$$

Where

$\mu_d = k_{dl} + k'[N]$

$$\mu_d = k_{dl} + \frac{k'k_1e^{aT}}{1 - k_1/k_2(1 - e^{aT})} \qquad ; \qquad V_o = V' + (V''-V')\frac{(T-nt_o)}{t_o}$$

⇩ Death due to lysozymes etc

⇩ Death due to neutrophils

[N] = concentration of neutrophils
$V_i$' = volume of the conjunctival tissue after inflammation
$k_1$ = initial concentration of neutrophils
$k_2$ = maximum concentration of neutrophils
a, k' = constant
T = time (assuming T=0 as the beginning of phase II)
V' = minimum volume of the tear film
V" = maximum volume of the tear film
$t_o$ = time taken for a tear drop to accumulate in the eye without falling
n = largest positive integer such that $T > nt_o$ OR number of teardrops fallen till time T

Substituting in equation (iii), we get

$$\frac{1}{V_i' + hV_0}\left[\left\{\mu_g - \left(k_{dl} + \frac{k'k_1e^{aT}}{1 - k_1/k_2(1 - e^{aT})}\right)\right\}V_i - hF_i\right] = \frac{1}{x}\frac{dx}{dt}$$

$$\Rightarrow \int_{T=0}^{T}\left[\frac{1}{V_i'+hV_0}\left[\left\{\mu_g - \left(k_{dl}+\frac{k'k_1e^{aT}}{1-k_1/k_2(1-e^{aT})}\right)\right\}V_i - hF_i\right]\right]dt = \int_{x=x_1}^{x}\frac{dx}{x}$$

On solving,

$$\frac{1}{V_{lm}}\left[\left(\mu_g V_i' - k_{dl}V_i' - hF_i\right)T - \frac{V_i'k'k_2}{a}\ln\left(1-\frac{k_1}{k_2}\left(1-e^{aT}\right)\right)\right] = \ln\left(\frac{x}{x_1}\right) \qquad \ldots (v)$$

Where $V_{lm}$ = log-mean volume = $\dfrac{h(V''-V')}{\ln\left(\dfrac{V_i'+hV''}{V_i'+hV'}\right)}$

$x_1$ = concentration of *S. aureus* which triggers immune reaction

In equation (v), T represents time from the beginning of the phase II. Hence, if t=0 represents time of inoculation, $T=t-t_1$; where $t_1$ is the time at which immune response is triggered.

Thus, $t_1 = \dfrac{V_i+hV'}{(\mu_g - k_{dl})V_i}\ln\left(\dfrac{x_1}{x_o}\right)$ … (vi)

Hence, the final equation comes out to be:

$$\mathbf{x} = \mathbf{x_o}\,\mathbf{exp}\left[\frac{(\mu_g - k_{dl})V_i}{V_i+hV'}t\right] \qquad \textbf{\textit{for } } x < x_1$$

$$= \mathbf{x_1}\,\mathbf{exp}\frac{1}{V_{lm}}\left[\left(\mu_g V_i' - k_{dl}V_i' - hF_i\right)T - \frac{V_i'k'k_2}{a}\ln\left(1-\frac{k_1}{k_2}\left(1-e^{aT}\right)\right)\right] \qquad \ldots(vii)$$

$$\textbf{\textit{for } } x > x_1$$

where

x = concentration of *S. aureus* in the tear film

h = fraction of cell concentration in the tear after filtration by epithilium

$x_o$ = initial concentration of *S. aureus* at the beginning of its logarithmic growth

$\mu_g$ = specific growth rate of *S. aureus*

$k_{dl}$ = specific death rate due to lysozymes, etc. (constant)

$x_1$ = concentration of *S. aureus* at which the immune response is triggered

$t_1$ = time at which the immune response is triggered

$k_1$ = initial basal concentration of neutrophils in the eye

$k_2$ = maximum level of neutrophils which are attained during the immune reaction

a, k' = constant

$F_i$ = volumetric feed rate of tears

$V_{lm}$ = log-mean volume = $\dfrac{h(V''-V')}{\ln\left(\dfrac{V_i'+hV''}{V_i'+hV'}\right)}$

## *3.2. Treatment using Antibiotics*

The primary treatment for bacterial conjunctivitis is the topical application of eye drops containing antibiotic. One of the most prescribed antibiotics for bacterial conjunctivitis is Alcon® Vigamox™ (Prescribed by ophthalmologist, Institute Hospital, IIT Madras). It is moxifloxacin hydrochloride ophthalmic solution 0.5% w/v. Moxifloxacin is an antibacterial derived from 8-methoxy fluoroquinolone. It acts by inhibiting topoisomerase II (DNA gyrase) and topoisomerase IV. The prescribed dose of the antibiotic was once every six hours (as per the Institute Hospital, IIT Madras). The antibiotic, being a very small molecule, is assumed to pass freely through the epithelial layer. Thus its concentration in the conjunctival tissue and the tear film is assumed to be same throughout. Also, the topical application of 1 drop is accompanied by the complete mixing of the antibiotic in the eye followed by release of a tear drop from the eye. Thus, the concentration of the antibiotic in the eye after falling of this tear drop is given by:

$$c_o = \frac{CV_d}{V_i'+V_d+V'} \qquad \text{…(viii)}$$

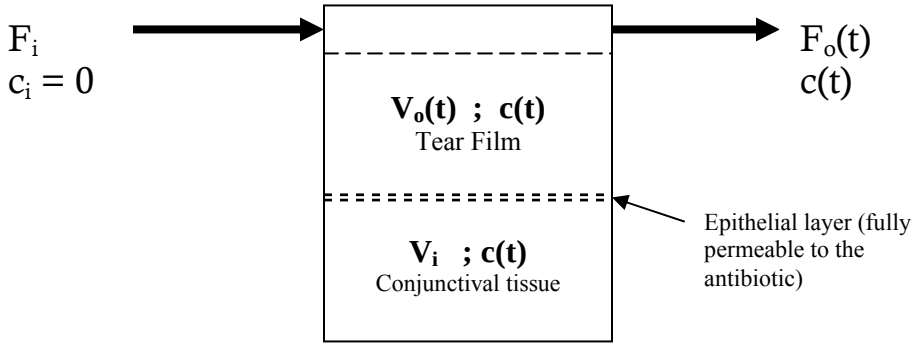
where

$c_o$ = concentration of antibiotic just after application and tear fall

C = concentration of antibiotic in the solution

$V_d$ = Volume of the drop applied

Assuming the above mentioned model for eye,



*Balance on antibiotic:*

$$\dot{I} + \dot{G} - \dot{C} - \dot{O} = \frac{dA}{dt}$$

$$\Rightarrow 0 + 0 - 0 - cF_o = \frac{d(cV)}{dt}$$

$$\Rightarrow - cF_o = c\frac{d(V_i + V_o)}{dt} + (V_i + V_o)\frac{dc}{dt}$$

$$\Rightarrow - cF_o = cF_i + (V_i + V_o)\frac{dc}{dt}$$

$$\Rightarrow \frac{(F_o + F_i)c}{(V_o + V_i)} = -\frac{dc}{dt}$$

$$\Rightarrow \int_{t=0}^{t} \frac{(F_o + F_i)}{(V_o + V_i)} dt = -\int_{c=c_o}^{c} \frac{dc}{c}$$

When integrated over a large interval, $F_o$ can be assumed to be constant and equal to $F_i$. Thus, solving

$$c = c_o e^{-\left(2F_i / V_{lm}\right)t} \qquad \ldots \text{(ix)}$$

The specific death rate of the bacterial cells due to antibiotic can be assumed to constant for concentrations much greater than the Minimum Inhibitory Concentration (MIC). Near the MIC, the specific death rate due to the presence of antibiotic varies linearly with the antibiotic concentration. Thus, the dependence of the specific death rate of bacterial cells on the antibiotic concentration can be assumed to follow a curve similar to the Michaelis - Menten enzyme kinetics.

$$\mu_d = k_{dl} + k'[N] + \frac{k_a c}{k_c + c} \qquad c = \text{concentration of antibiotic}$$

Substituting in equation (iii),

$$\frac{1}{V_i' + hV_0}\left[\left\{\mu_g - \left(k_{dl} + \frac{k' k_1 e^{aT}}{1 - k_1/k_2 (1 - e^{aT})} + \frac{k_a c_o e^{-\left(2F_i / V_{lm}\right)T}}{k_c + c_o e^{-\left(2F_i / V_{lm}\right)T}}\right)\right\} V_i - hF_i\right] = \frac{1}{x}\frac{dx}{dt}$$

$$\Rightarrow \int_{T=0}^{T}\left[\frac{1}{V_i' + hV_0}\left[\left\{\mu_g - \left(k_{dl} + \frac{k' k_1 e^{aT}}{1 - k_1/k_2 (1 - e^{aT})} + \frac{k_a c_o e^{-\left(2F_i / V_{lm}\right)T}}{k_c + c_o e^{-\left(2F_i / V_{lm}\right)T}}\right)\right\} V_i - hF_i\right]\right] dt = \int_{x=x_1}^{x} \frac{dx}{x}$$

On integrating, we get,

$$\frac{1}{V_{lm}}\left[\left(\mu_g V_i' - k_{dl} V_i' - hF_i\right)T - \frac{V_i' k' k_2}{a}\ln\left(1 - \frac{k_1}{k_2}\left(1 - e^{aT}\right)\right) - \frac{k_a V_{lm}}{2F_i}\ln\left(\frac{k_c + c_o}{k_c + c_o e^{\left(-2F_i/V_{lm}\right)T}}\right)\right] = \ln\left(\frac{x}{x'}\right)$$

… (x)

Where x' is the concentration of *S. aureus* at the time of topical application of antibiotic.

The above equation is valid for the time between two consecutive applications of antibacterial eye drops (6 hours in this case). After 6 hours, the concentration of antibiotic can be assumed to be zero, thus the concentration of antibiotic in the eye after the next consecutive application can be assumed to be independent of the amount remnant from last application.

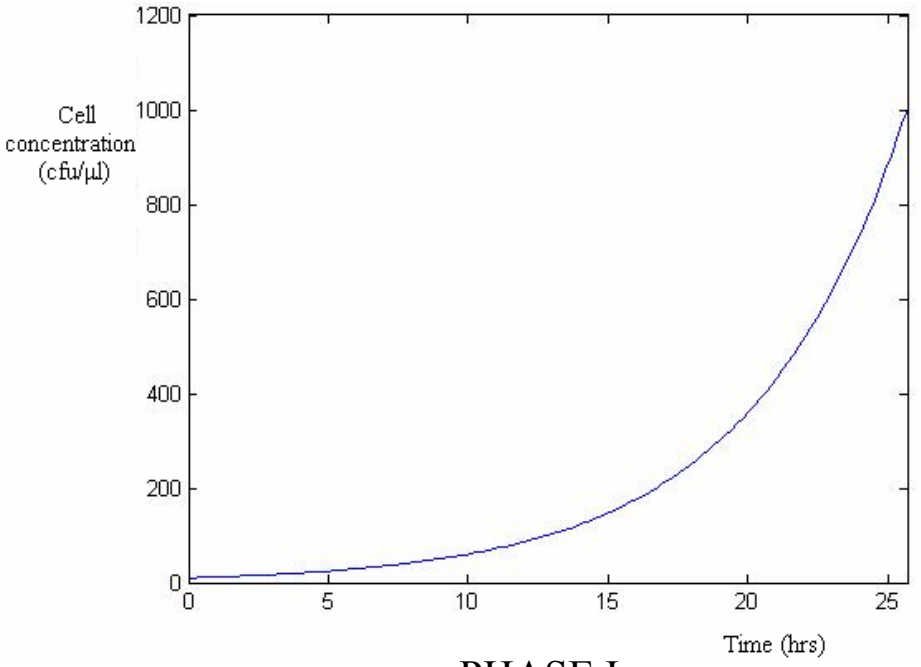
# 4. RESULTS AND DISCUSSION

## Estimation of Parameters

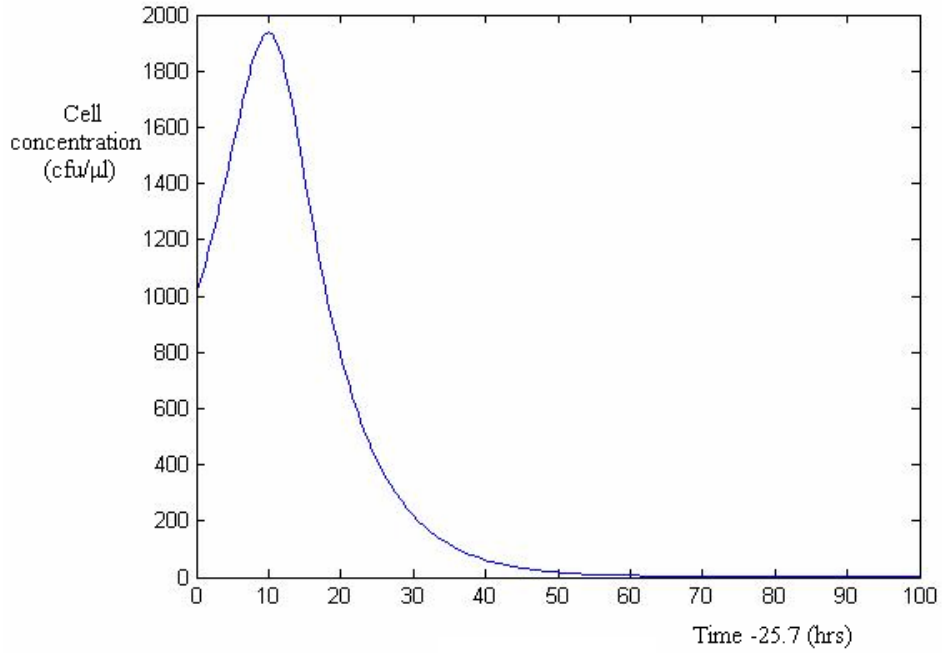
{**NOTE** - The parentheses shows the basis for assumption of the value}

- $x_o$ = 10 cfu/µl (assumed)
- $\mu_g$ = 0.2 $hr^{-1}$ (**[7]** Table 1; assuming the values to be valid in tears also)
- $k_{dl}$ = one-tenth of growth rate, i.e. 0.02 $hr^{-1}$ (**[8]**)
- h = 0.03 (assumed low value due to the presence of tight junctions in the epithelial layer)
- $x_1 = 10^3$ cfu/µl (**[9]** Table 1; assuming the values to be valid in tears also)
- $k_1$ = 4.8 neutrophils/µl (blood test report of Abhishek Tiwari previous to the infection)
- $k_2 = 10^3$ neutrophils/µl (**[10])**
- a = 0.5 $hr^{-1}$(assumed, considering the case of infection in Abhishek Tiwari)
- k' = $2.1875 \times 10^{-7}$ $\mu l^{-1} hr^{-1}$(assumed, considering the case of infection in Abhishek Tiwari)
- $V_i$ = 50 µl; $V_i'$ = 80 µl (interpreted using sources on the world wide web **[11]**)
- V' = 7 µl ; V" = 27 µl (**[12]**)
- $t_o$ = 5 min. (average time of tear drop fall observed in the case of conjunctivitis of Abhishek Tiwari)
- $F_i$ = 4 µl/min = 240 µl/hr (calculating from above data)

Substituting the above parameters in the final equation, the corresponding concentration of *S. aureus* (cfu/µl) can be plotted against time (hrs) [Refer page 8]. The time required for the cell concentration to reach a value of 1cfu/µl is calculated to be 98 hours (approximately). **Thus the time required by the body mechanisms for the eradication of *S. aureus* from the conjunctival tissue of the eye is calculated to be approximately 98 hours, starting from the time of infection.**




PHASE I




PHASE II

In the model proposed for treatment using antibiotics, the value of all the parameters of natural immune response and growth rates of *S. aureus* are assumed to be unaffected by the presence of antibiotics. The value of $k_a$ is assumed to be 1 hr-1 (assumption based on Fig.1 **[13]**). The value of $k_c$ is assumed to be same as the MIC, i.e 0.06 µg/ml. Substituting the above values in equation (x), we get that the bacterial concentration falls to a value of 1 cfu/µl after 65 hours (approximately). (This value is calculated assuming the antibiotic treatment is started after 2 hours of infection).

## 5. CONCLUSION

The eye has been modeled as fed-batch reactor with an internal filter. The time required by the body responses to eradicate the pathogenic bacteria (*S. aureus*) has been calculated to be approximately 98 hours starting from the time of infection. This time is significantly reduced (65 hours) if the body mechanism is supported by topical application of Alcon® Vigamox™ (0.5% w/v moxifloxacin) at the frequency of 4 drops per day. Thus we can say that antibiotic treatment for acute bacterial conjunctivitis is just a matter of speed and not need.

## 6. LIMITATION AND SCOPE FOR IMPROVEMENT

In the above model, it is assumed that the lacrimation rate does not change while sleeping. Thus, the accuracy of the model can be improved by taking the variation of lacrimation rate due to sleeping.

It is also assumed that the infected person does not wash his/her eyes during the infection.

The variation of lacrimation rate and conjunctival volume as a function of time is neglected and an average value for these parameters after the immune response is taken into account. This was done due to lack of sufficient information on the time-dependence of these parameters. Thus, the accuracy of this model can be further improved by taking into account, the time dependent variation of these parameters.

# 6. ACKNOWLEDGEMENTS

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