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Sonora, México

Effect of the superficial gas velocity on hydrodynamics and growth kinetics of *Steinernema spp.* in a bubble column

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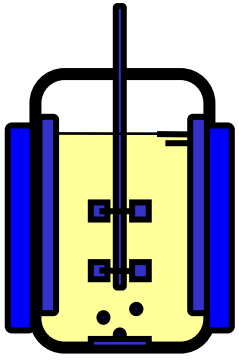
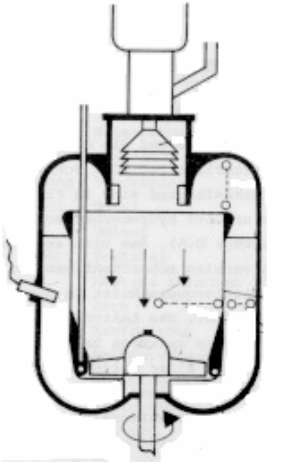
Bioreactors used for EPN mass production in liquid culture

According to the main energy input

Mechanical agitation



Stirred TanK Reactor



Compact Propeller Loop Reactor

Pneumatic agitation

Bubble column, airlift

Bioreactor type and operation conditions affect the yield of EPN

- ✓Shear forces (liquid and gas movement)
- ✓Oxygen transfer rate (*Xenorhabdus requires more O_2 than EPN*)
- ✓Mating

Shear forces (Turbulence)

Shear forces from 67 to 450 per s did not harm adult females. Only J2 died.

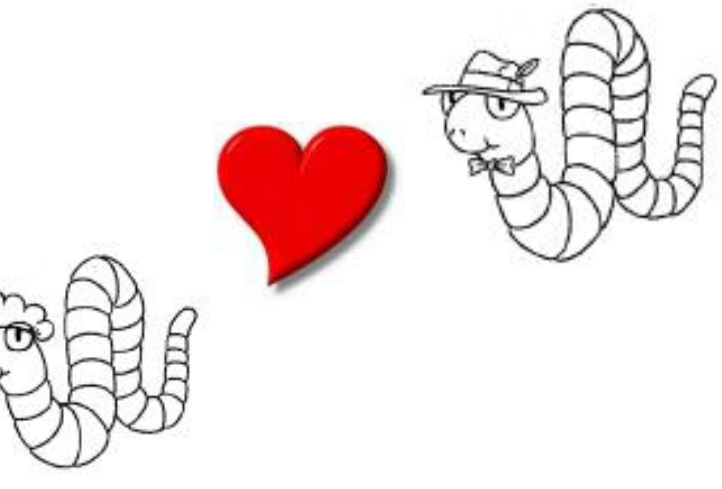
Although in STR and pneumatic reactors used for EPN mass production, shear forces are $\leq 100\ s^{-1}$

Chavarría, 2001

Oxygen Transfer (K_La)

Pneumatic bioreactor	Stirred bioreactor
k_La from 0.003 to 0.005 per second	k_La of 0.096 per second
102,000 nematode/ml	23,000 nematode/ml

Contact sexual of male and female is neccesary



Is mating prevent by turbulence?

Bioreactor and operation conditions

⇩

Allow nematode´s successful copulation

⇩

Reproduction

⇩

High concentrations of nematodes

Are bubble columns an option for EPN mass production?

YES

Shear forces ↓ Oxygen Transfer ↑

This effects probably can allow the succesfull copulation

and

bubble columns are the most energy efficient reactors and the cheapest

Bubble Column
High = 45 cm, ø = 5 cm

Superficial gas velocity (Vg)

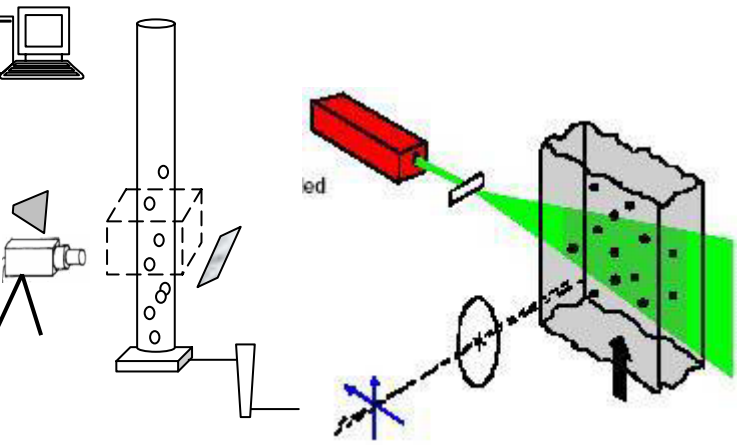
0.3 cm/s

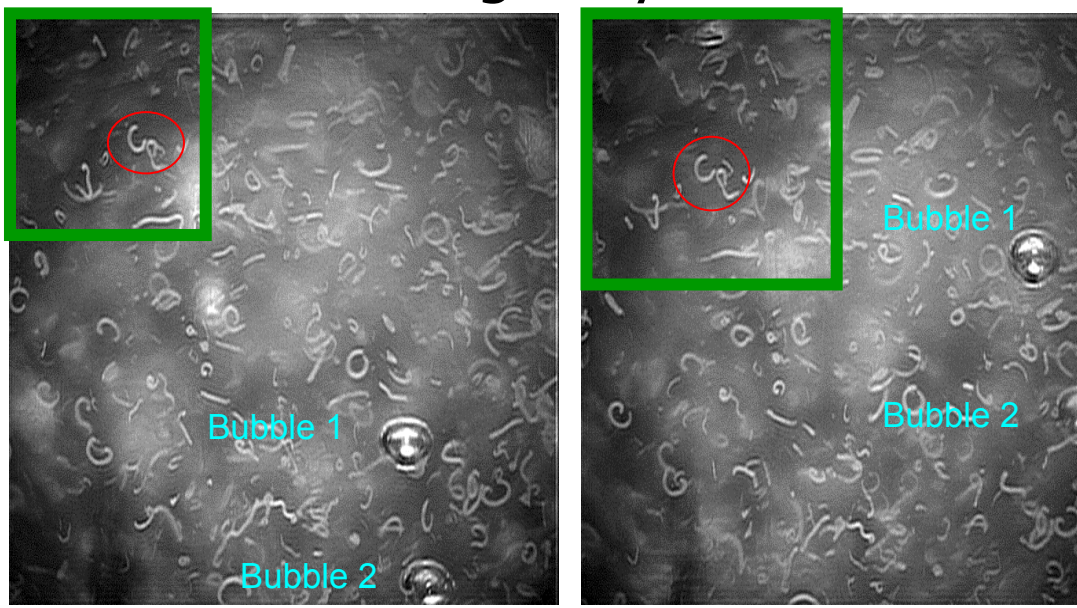
or

0.85 cm/s

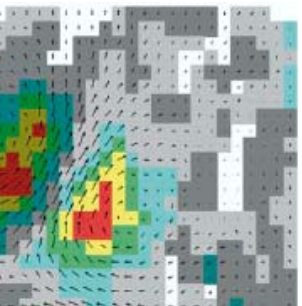
Instantaneous Velocities calculated with **P**article **T**racking **V**elocimetry

30 images by second





T_n T_{n+1}



- Flow pattern

$$v = \frac{\text{distance}}{\text{time}}$$

Stamhuis EJ et al. 2002

Nematodes and gas velocities

Superficial gas velocity (cm/s)	Maximum reproduction factor $(C/Co)_{max}$	Velocity of liquid (cm/s)	Velocity of gas (cm/s)
0.3	100.91	3.9 (0.23) a	15.9 (0.94) b
0.85	151.61	5.3 (0.35) a	13.3 (0.87) b

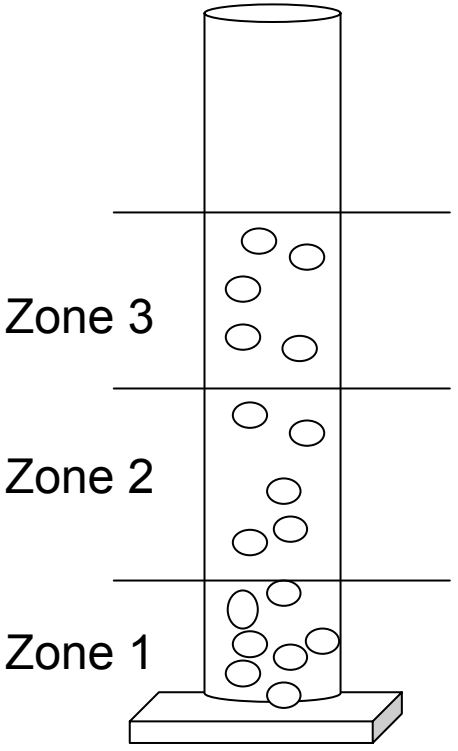
Velocities were not different at both V_g.
Reproduction factor is highest at the highest V_g

Oxygen Transfer

Superficial gas velocity (cm/s)	Maximum reproduction factor $(C/Co)_{max}$	k_La (s^{-1})
0.3	100.91	0.111
0.85	151.61	0.173

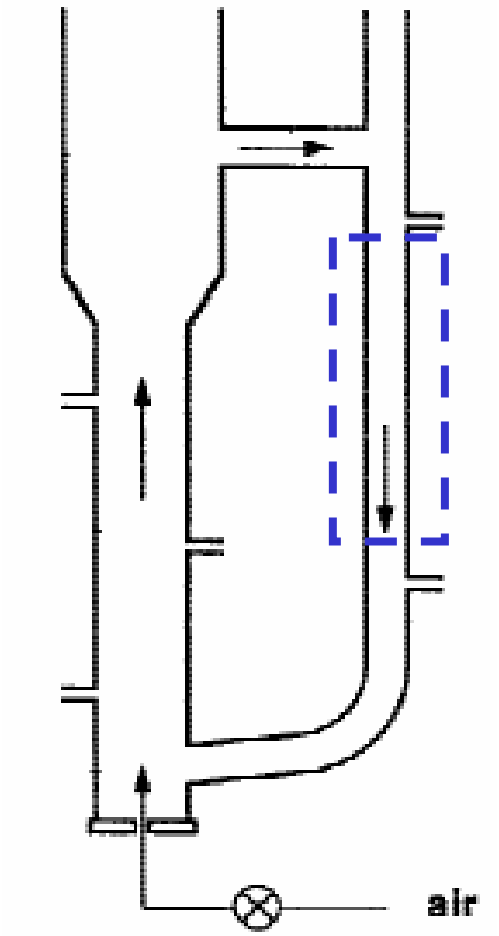
Both, reproduction factor and k_La were highest at Vg = 0.85

Distribution of females



Bubble Column	Females %	
	Vg = 0.3 cm s^{-1}	Vg = 0.85 cm s^{-1}
Zone 1	$50.2_{(5)}{}^{a}$	$50.3_{(9)}{}^{a}$
Zone 2	$45.8_{(4)}{}^{b}$	$44.2_{(7)}{}^{b}$
Zone 3	$37.4_{(6)}{}^{c}$	$40.2_{(6)}{}^{c}$

Accumulation of females in an external airlift bioreactor



The highest EPN yield at the lowest airflow rate suggested that the increase in mating rate was due to a lower liquid circulation velocity that favor accumulation of females and therefore mating

Neves JM., et al. 2001

Which are the critical factors in the bioreactor for EPN mass production?

Engineering challenges

- ✔ Shear forces that not affect nematodes
- ✔ Oxygen transfer efficient
- ✔ Heterogeneous distribution of adults

Biology

- ? Mating behavior and chemical signals

Is mating behavior chemically mediated or it is a result from incidental encounters?

Sex attraction has been described in some species of nematodes but has not yet for steinernematids

We are stablishing a bioessay system to study the mating behavior and get chemical signals

Lewis EE.,et al, 2002

Sexual behavior in *Steinernema spp*.

T = 0 min

T = 1 min

T = 2 min

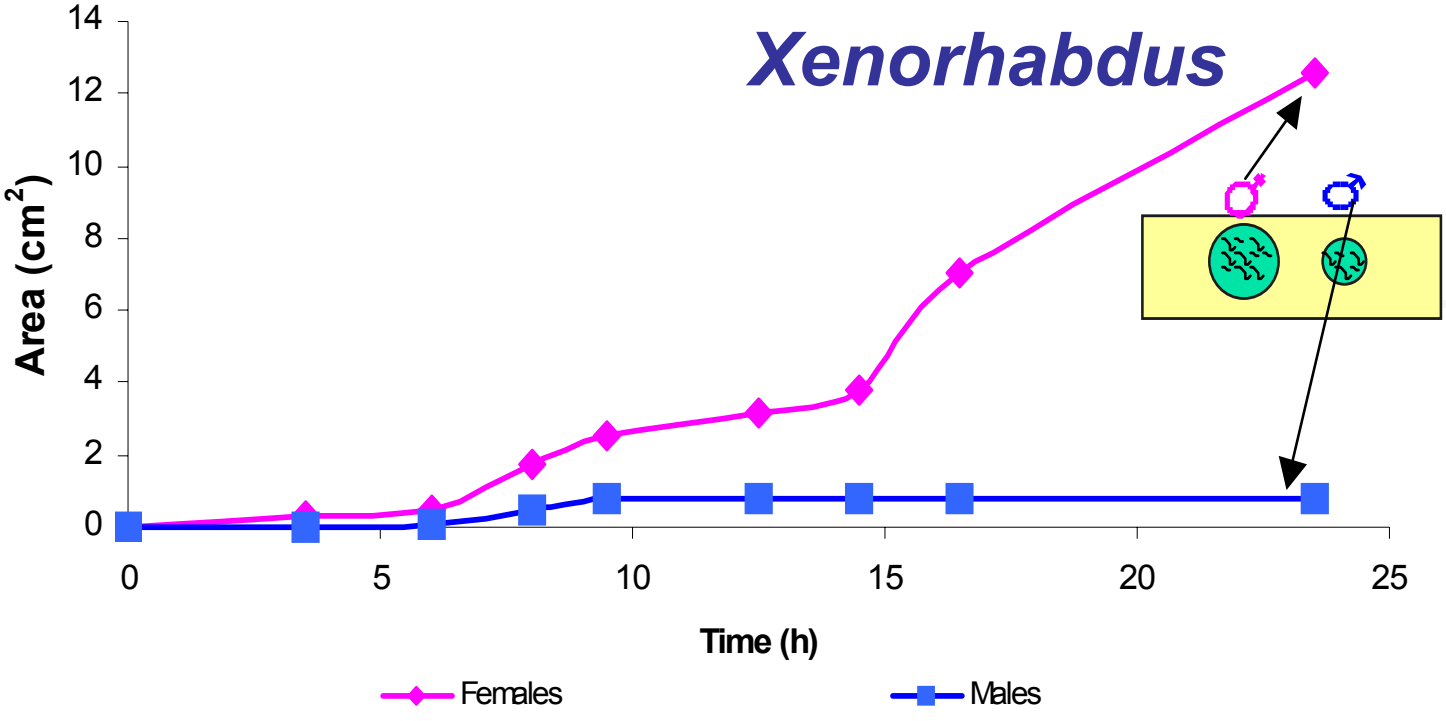
T = 3 min

Male was near to female and curled around female´s body and there was copulation

T = 4 min

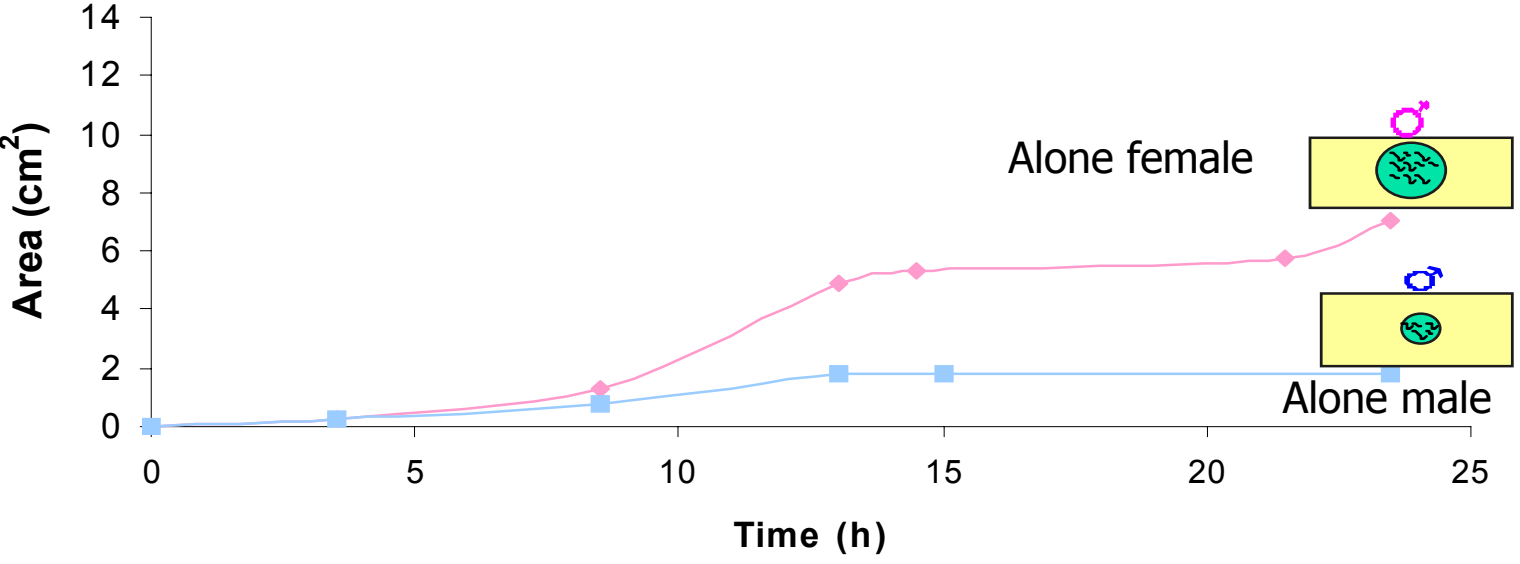
T = 7 min

Movement of nematodes in a slide with *Xenorhabdus*



Together females and males

Females move more than males when they are together than when they are alone



Challenges

- Is mating mediated by chemical signals in *Steinernema*?
- Is the mating behavior important in liquid bioreactors?

Thanks