

Photo-Induced Antibacterial Agents and Potential Applications

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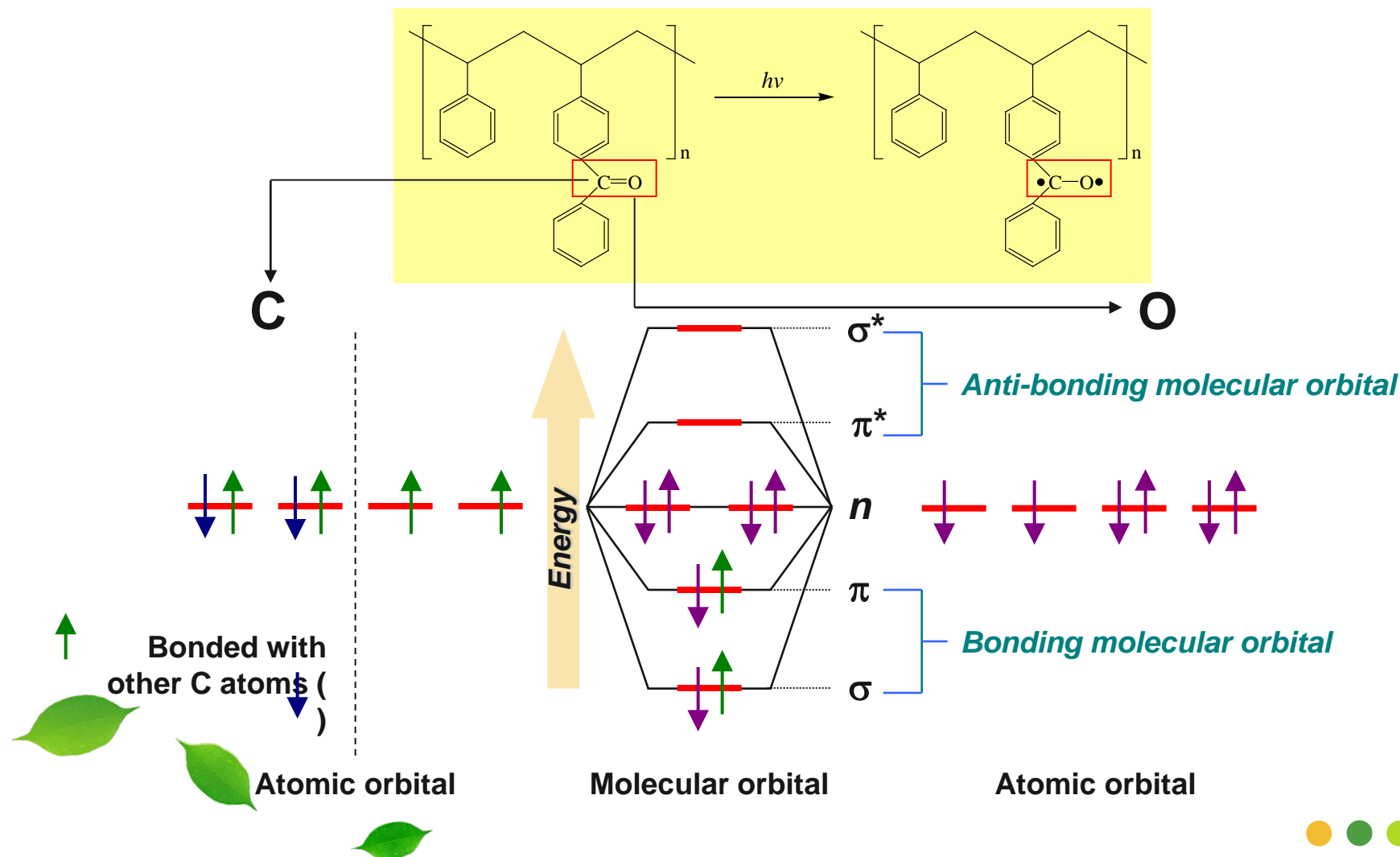


Introduction

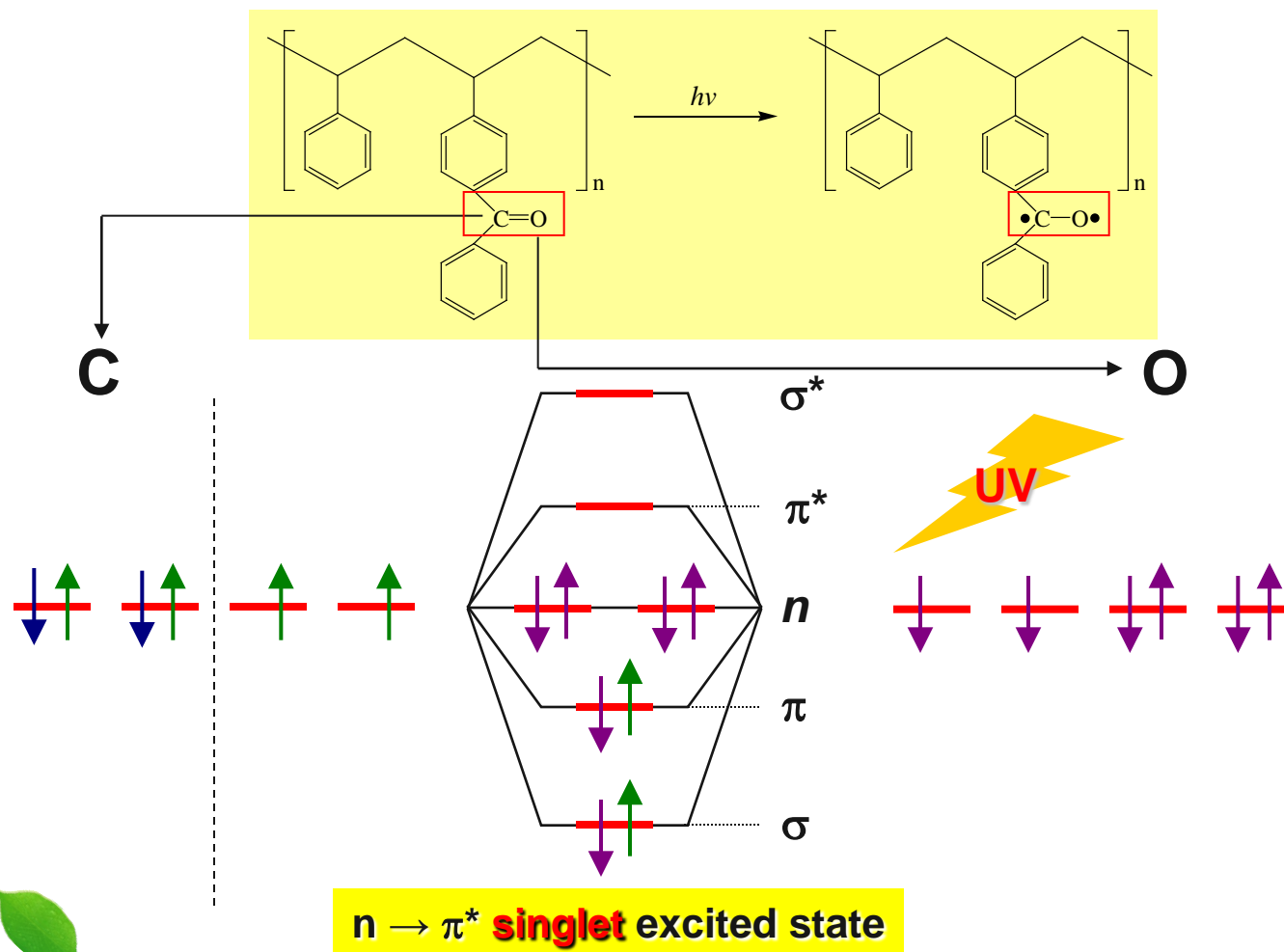
- Photo-active compounds such as benzophenone and certain acid dyes could become antimicrobial when exposed to UVA and even fluorescent light. When these compounds are properly incorporated onto surfaces polymers or fibers, they are able to provide antimicrobial functions as well with exposure to light.
- The antimicrobial functions of the compounds attribute to generation of radicals, which lead to formation of reactive species that could kill microorganisms.
- Since hydrogen peroxide could be formed with moisture on these surfaces, antimicrobial functions can be observed without of light.



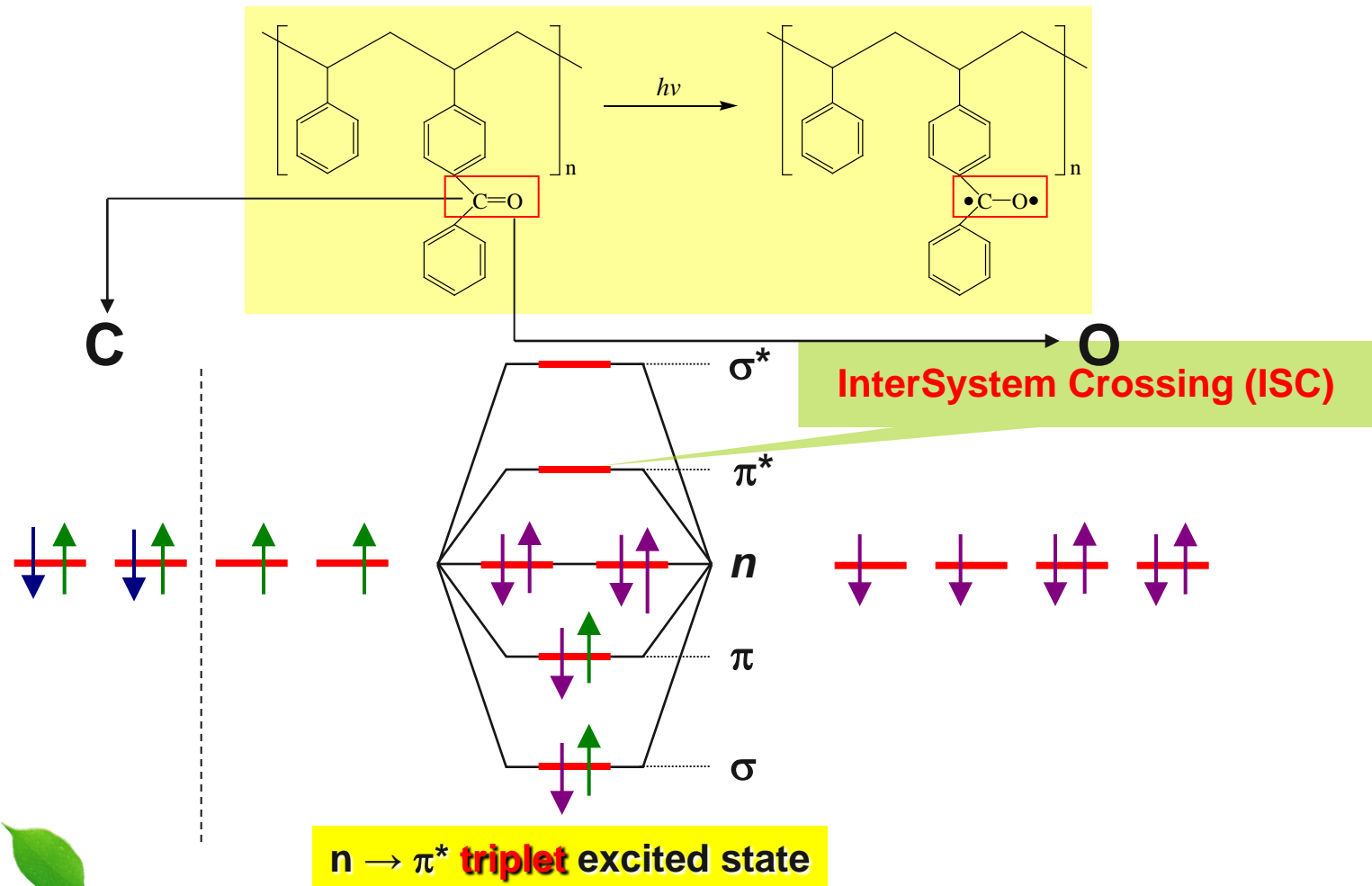
Introduction



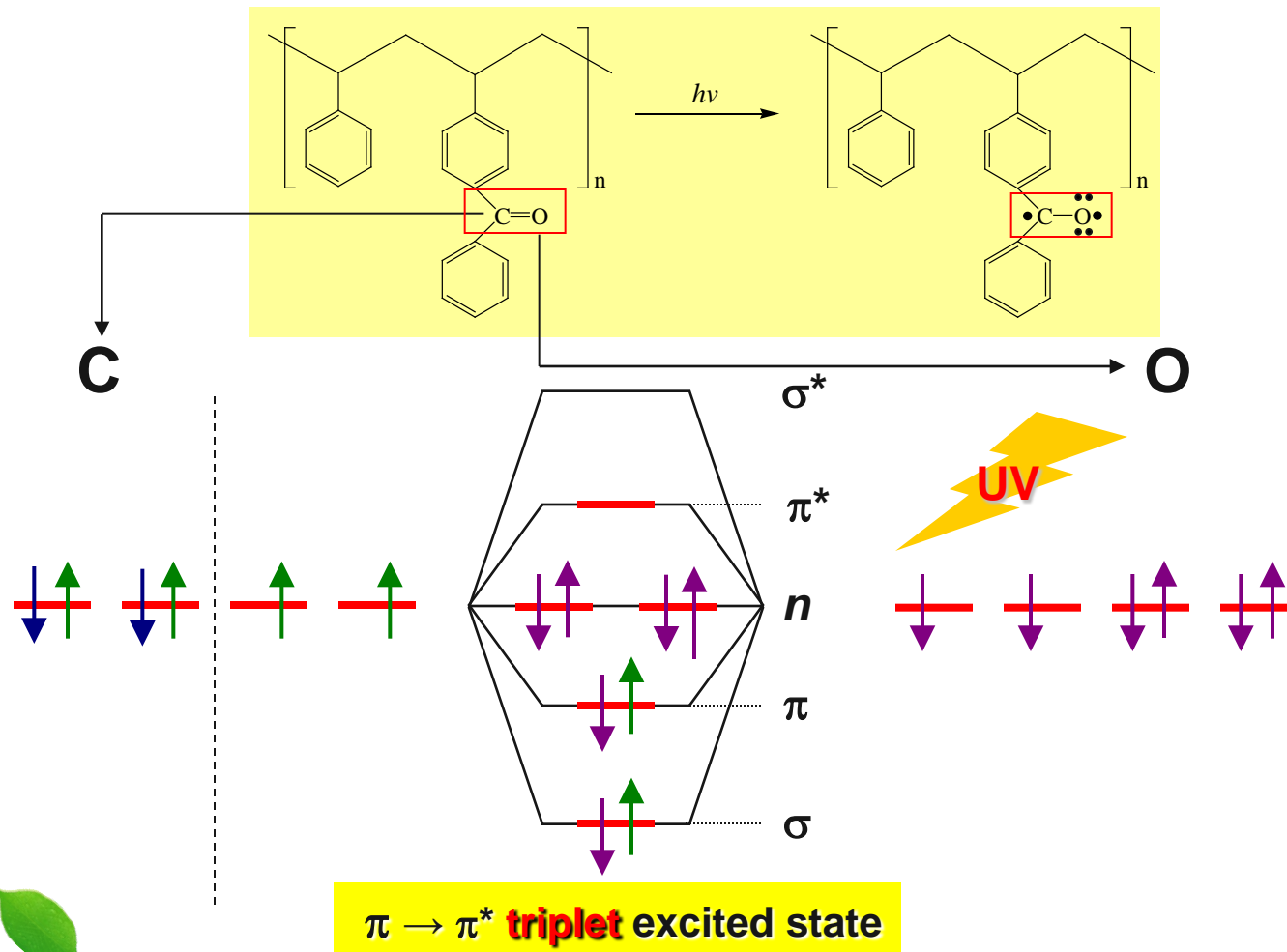
Introduction



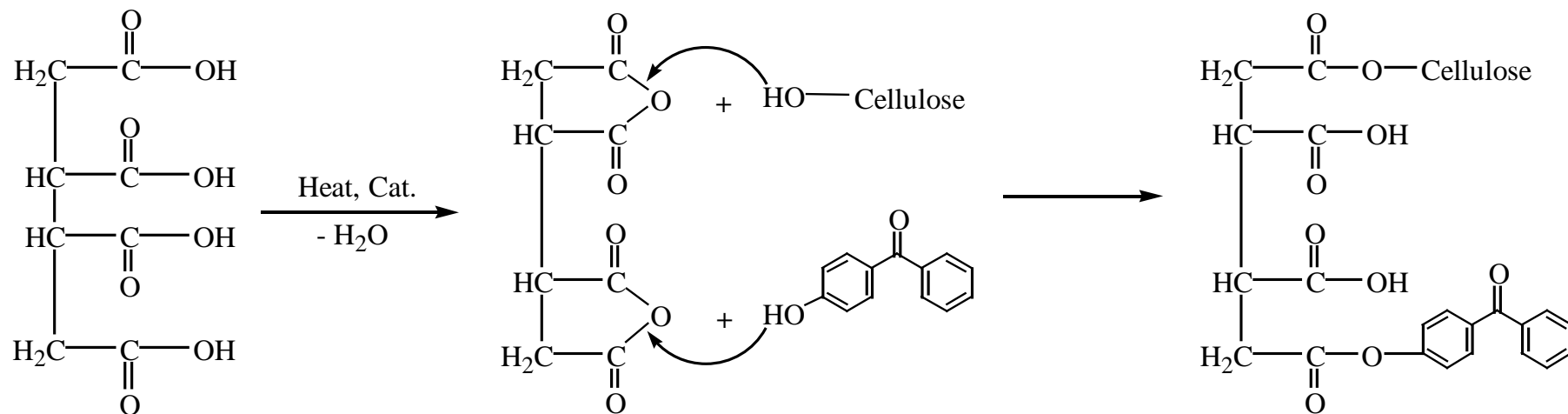
INTRODUCTION



INTRODUCTION



EXPERIMENTS



Scheme 1. Incorporation mechanism of benzophenone chromophoric group to cotton fabrics.

Fabric: Desized cotton print cloth (Testfabrics, Inc., No.400)

Benzophenone derivative: 4-hydroxybenzophenone (Aldrich Co, USA)

Crosslinker: BTCA (Aldrich Co, USA)

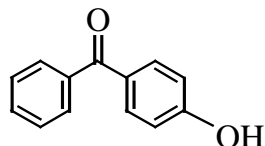
Catalyst: sodium hypophosphite hydrate (Aldrich Co, USA)

RESULTS

Colony numbers of *S. aureus* and *E. coli* after injection of 0.1mL bacteria suspension on agar plates and incubation at 37°C for 18h.

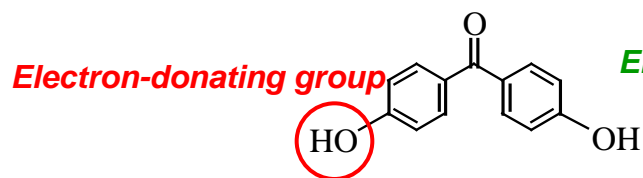
	Dilution ratio of the <i>S. aureus</i> solution after contact time				Dilution ratio of the <i>E.coli</i> solution after contact time			
	× 10	× 10 ²	× 10 ³	× 10 ⁴	× 10	× 10 ²	× 10 ³	× 10 ⁴
[UV exposed] Pristine cotton fabrics	∞	57	5	0	∞	∞	∞	561
[No_UV exposure] 0.1M 4-hydroxybenzophenone treated cotton fabrics	∞	12	1	0	∞	∞	∞	47
[UV exposed] 0.1M 4-hydroxybenzophenone treated cotton fabrics	0	0	0	0	8	0	0	0

Benzophenone Derivatives

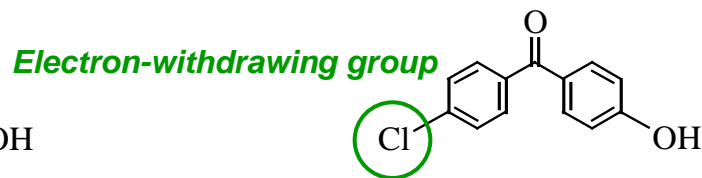


4-hydroxybenzophenone
(**4-HBP**)

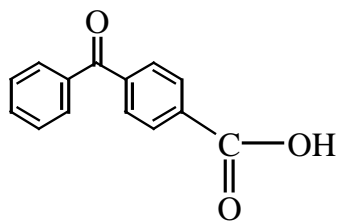
With BTCA



4,4'-dihydroxybenzophenone
(**44'-DHBP**)



4-chloro-4'-hydroxybenzophenone
(**4C4'HBP**)



4-benzoylbenzoic acid
(**4-BBA**)

Without BTCA


RESULTS

Table 2. Colony numbers of *S. aureus* after placement of 0.1mL bacteria suspension on agar plates and incubation at 37°C for 18h.

Sample		$\times 10$	$\times 10^2$	$\times 10^3$	$\times 10^4$	Reduction %
Pristine	No-UV	∞	∞	∞	285	-
	UV	∞	∞	∞	230	-
4-HBP	No-UV	∞	∞	∞	47	83.509
	UV	0	0	0	0	>99.999
4-HBP (after 10h UV)	No-UV	∞	∞	∞	62	78.246
	UV	0	0	0	0	>99.999
4,4'-DHBP	No-UV	∞	64	5	0	99.825
	UV	4	0	0	0	99.998
4-BBA	No-UV	∞	∞	∞	77	72.982
	UV	∞	∞	∞	26	88.696
4C4'HBP	No-UV	∞	∞	146	31	89.123
	UV	184	21	0	0	99.909

Photo-Active Dyes

**2,6-anthraquinone
disulfonate (2,6-AQS)**



**2-anthraquinone
sulfonate (2-AQS)**

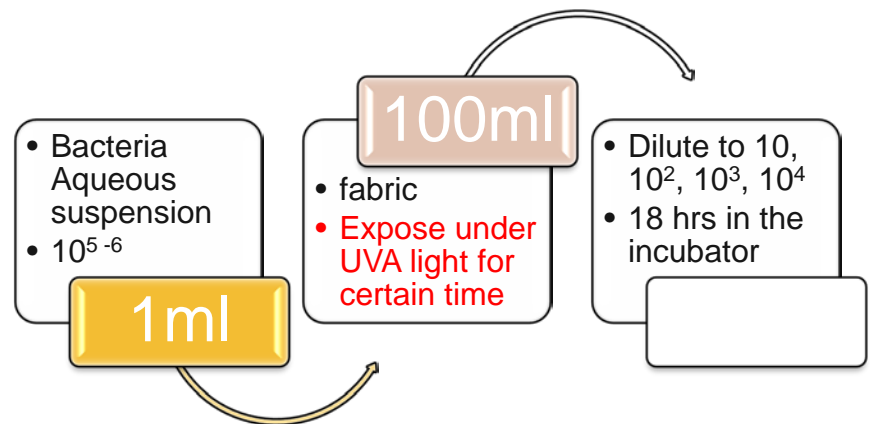
**2,7-anthraquinone
disulfonate (2,7-AQS)**



Protocols

Immobilization process

Modified AATCC 100 (2004)

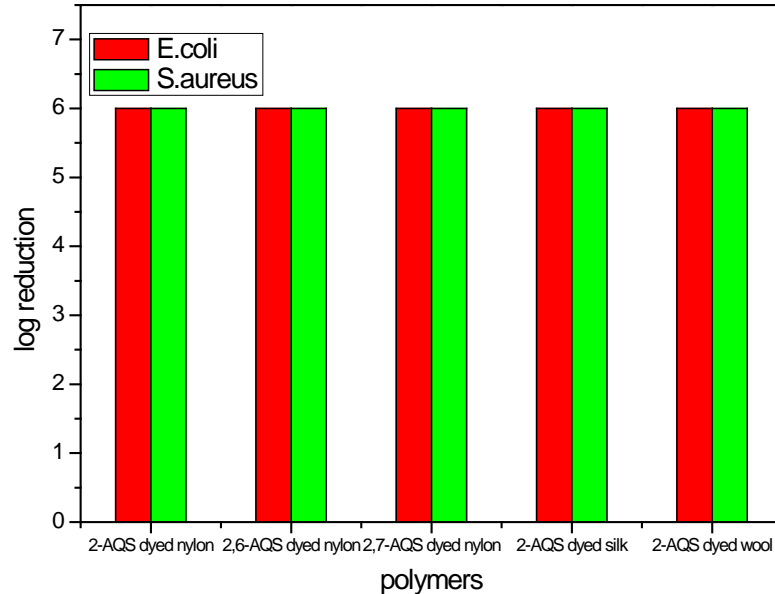


Normal acid dyeing process

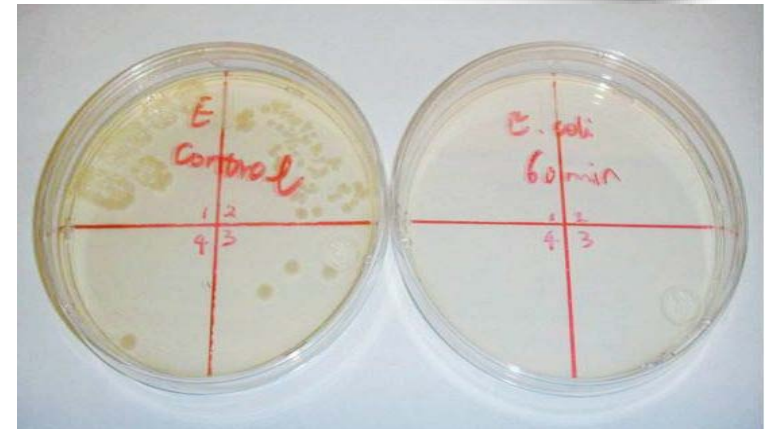
Control Sample :
Untreated Sample
Dyed sample in Dark condition
Bacteria : *E.coli* *S.aureus*



Biocidal Functions of Dyed Fabrics in Different Concentrations



Dye concentration: 1.5% (owf)
 UVA (365 nm) Exposure time: 1hr
 Bacteria Concentration : $\sim 10^6$ cfu/mL

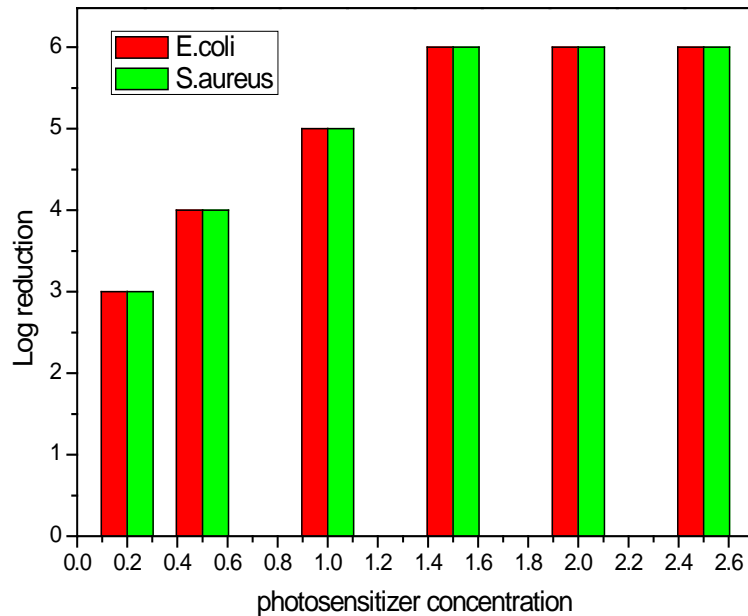


Control

2-AQS treated Nylon 66
 fabric



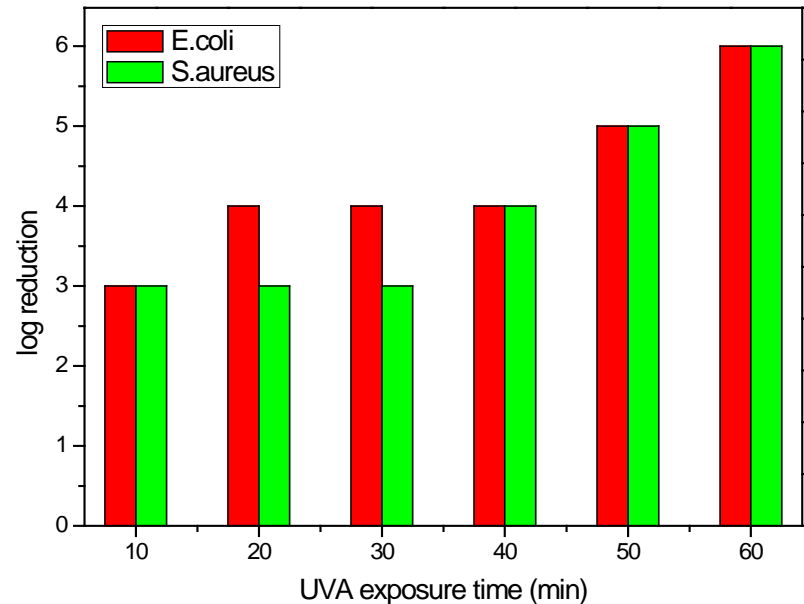
Biocidal Functions of Dyed Fabrics under UVA



UVA exposure time: 1hr

Dye compound: 2-AQS

Bacteria concentration: $\sim 10^6$ cfu/mL



Dye concentration: 1.5%(owf)

Dye compound : 2-AQS

Bacteria concentration : $\sim 10^6$ cfu/mL

UVA (365 nm) five 8 watts lamps



Biocidal Functions under Fluorescent Light

Nylon 66	1.5 % 2-AQS	1.5 % 2,6-AQS	1.5 % 2,7-AQS	2.0 % 2-AQS	2.5 % 2-AQS
Reduction of E.coli	96.2%	95.7%	64.6%	99.4%	99.9%
Reduction of S.aureus	97.6%	96.3%	99.4%	99.9%	99.9%

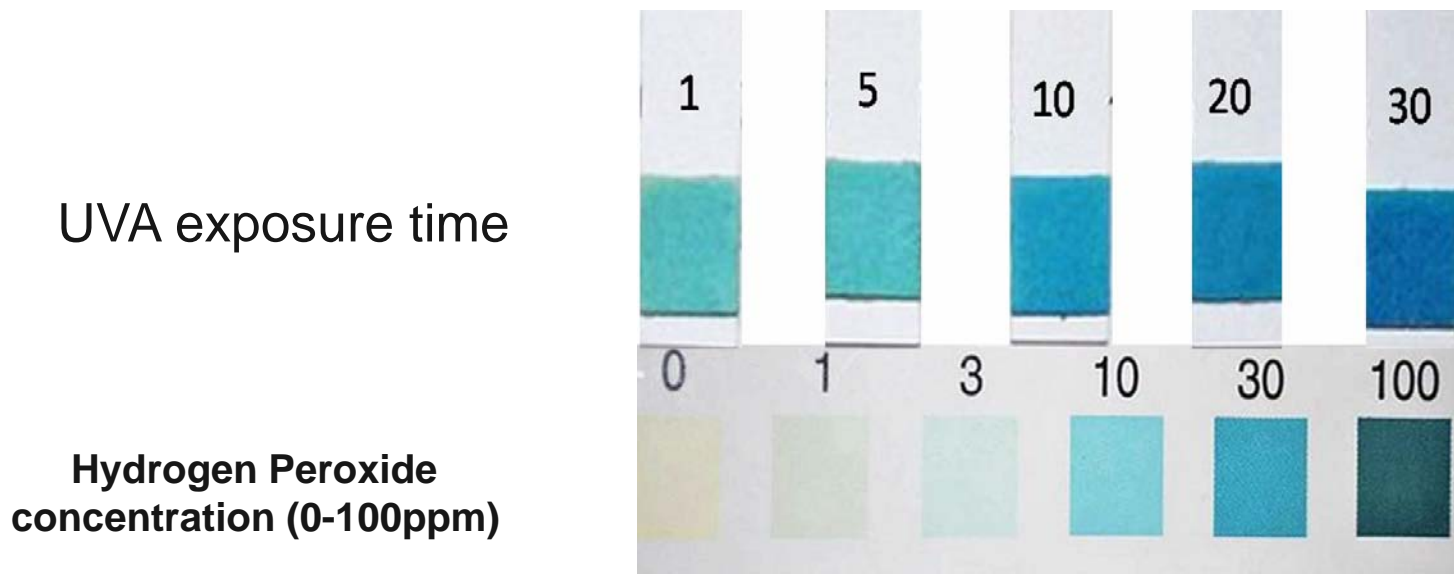
Light exposure time :1 hr

Light source: Five 8 w fluorescent lamps

Bacteria concentration : $\sim 10^6$ cfu/mL



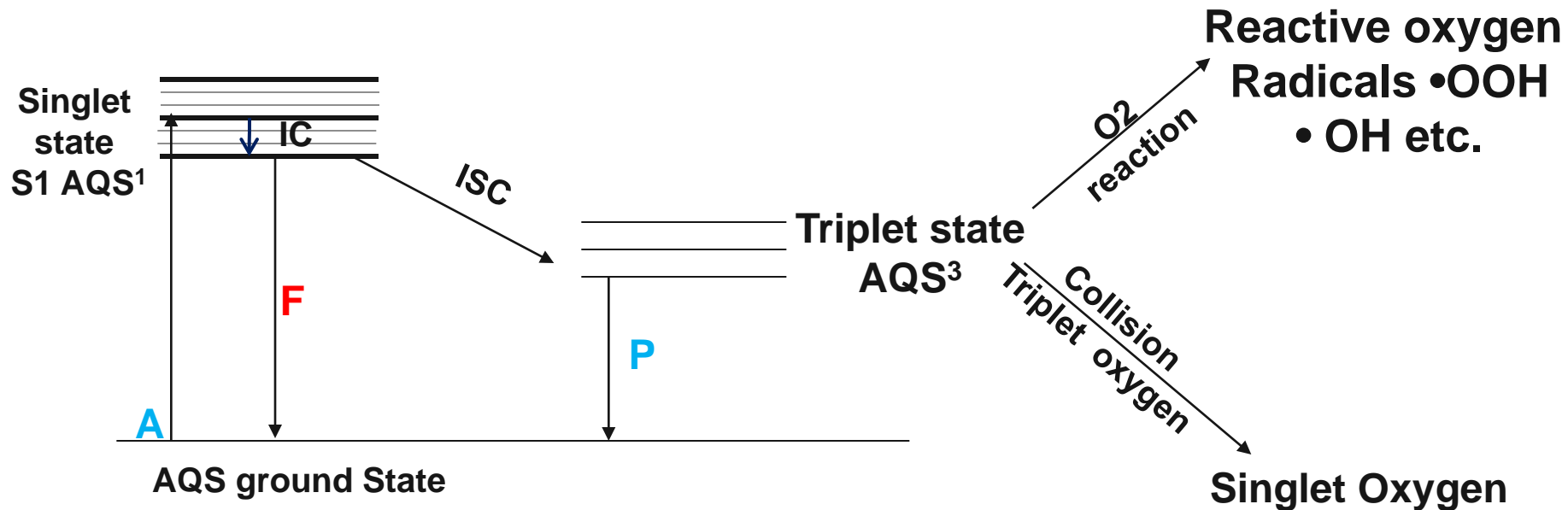
Formation of Hydrogen Peroxide



Formation of hydrogen peroxide of 2-AQS dyed Nylon 66 fabric exposed to UVA light for 1 min, 5 min, 10 min, 20 min, and 30 min, respectively



Photo-activation Process



A: Photon Absorption

F: Fluorescent light

P: Phosphorescent light

IC: Internal Conversion

ISC: intersystem crossing

Triplet oxygen: ground state oxygen



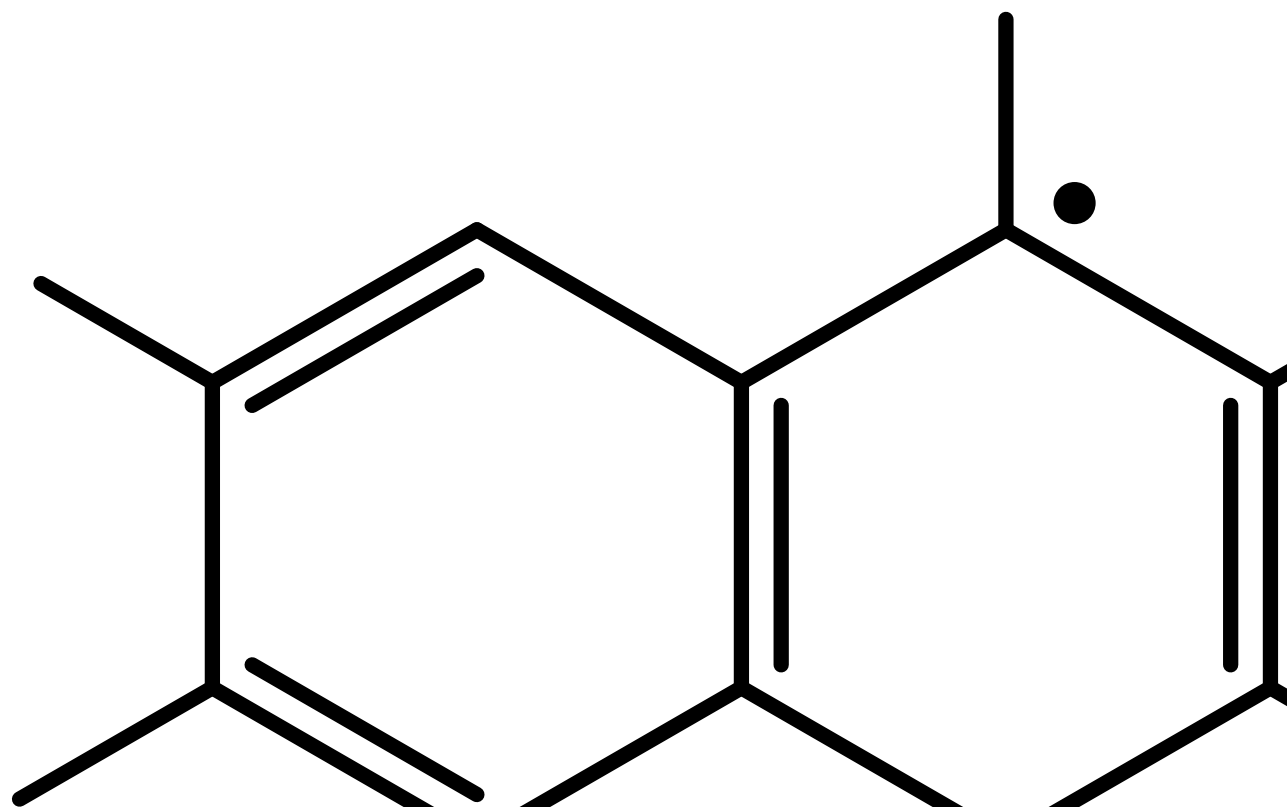
Photochemistry Mechanism

R- H, or SO_3Na

R'-could be any group

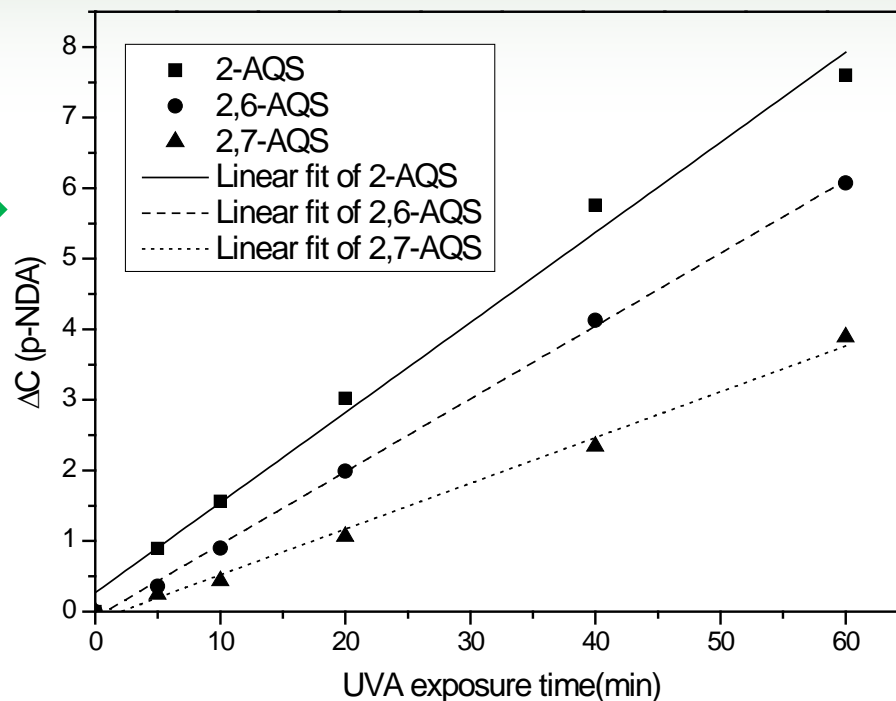


Photochemistry Mechanism



Formed Reactive Oxygen Species

Quantification of hydroxyl radicals produced by 2-AQS, 2,6-AQS, 2,7-AQS under different UVA exposure times



Quantum yields of singlet oxygen of 2-AQS and Rose Bengal



	Singlet oxygen quantum yield	Solvents (volume)
Rose Bengal with L-histidine	0.76	90%Water, 10% ethanol
2-AQS with L-histidine	1.87	90%Water, 10% ethanol



PU Coating Results

	BP added coating solutions				
	BP-0	BP-1	BP-2	BP-3	BP-4
PU based coating solution (g)	5	5	5	5	5
BP/n-propanol (0.15g/mL; 15.733 wt%) (g)	0	0.0625	0.125	0.25	0.5
n-propanol (g)	0.5	0.4375	0.375	0.25	0
BP concentration (wt%)	0	0.179	0.358	0.715	1.430
	RB added coating solutions				
	RB-0	RB-1	RB-2	RB-3	RB-4
PU based coating solution (g)	5	5	5	5	5
RB/n-propanol (0.15g/mL; 15.733 wt%) (g)	0	0.0625	0.125	0.25	0.5
n-propanol (g)	0.5	0.4375	0.375	0.25	0
RB concentration (wt%)	0	0.179	0.358	0.715	1.430



Antimicrobial Results of BP (UVA 365nm)

<i>E.coli</i>	Dilution Ratio of Bacteria Solution after Exposure Time of 1.5 hr				Reduction of Bacteria (%)
	10	10 ²	10 ³	10 ⁴	
Blank	∞	∞	∞	26	-
BP-0	∞	19	3	1	-
BP-1	∞	17	4	0	0
BP-2	∞	28	1	0	0
BP-3	87	6	2	0	54.21
BP-4	1	0	0	0	99.47

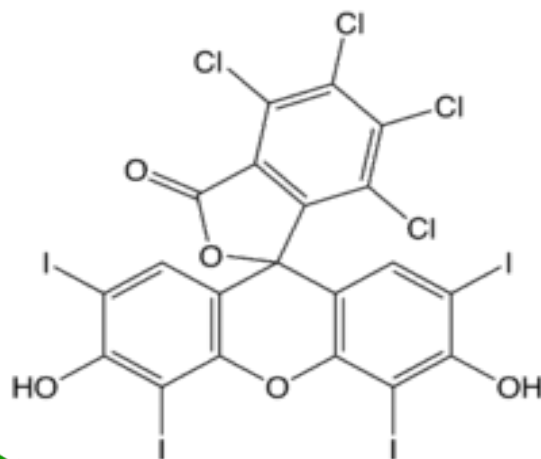
<i>S.aureus</i>	Dilution Ratio of Bacteria Solution after Exposure Time of 1.5 hr				Reduction of Bacteria (%)
	10	10 ²	10 ³	10 ⁴	
Blank	∞	∞	∞	∞	-
BP-0	32	6	0	0	-
BP-1	19	3	0	0	40.63
BP-2	7	1	0	0	78.13
BP-3	0	0	0	0	>99.9999
BP-4	0	0	0	0	>99.9999

Antimicrobial Results of RB (UVA 365 nm)

<i>E.coli</i>	Dilution Ratio of Bacteria Solution after Exposure Time of 1.5 hr				Reduction of Bac teria (%)
	10	10 ²	10 ³	10 ⁴	
Blank	∞	∞	∞	26	-
RB-0	∞	19	3	1	-
RB-1	∞	21	2	0	0
RB-2	∞	13	1	0	31.58
RB-3	∞	7	0	0	63.16
RB-4	0	0	0	0	>99.9999
<i>S.aureus</i>	Dilution Ratio of Bacteria Solution after Exposure Time of 1.5 hr				Reduction of Bac teria (%)
	10	10 ²	10 ³	10 ⁴	
Blank	∞	∞	∞	∞	-
RB-0	32	6	0	0	-
RB-1	11	1	0	0	65.63
RB-2	4	0	0	0	87.50
RB-3	0	0	0	0	>99.9999
RB-4	0	0	0	0	>99.9999

Antimicrobial Results under Fluorescent Light

<i>E.coli</i>	Dilution Ratio of Bacteria Solution after Exposure Time of 1.5 hr				Reduction of Bacteria (%)
	10	10 ²	10 ³	10 ⁴	
Blank	∞	∞	164	21	-
BP-0 or RB-0	∞	79	7	3	-
BP-4	0	0	0	0	>99.9999
RB-4	0	0	0	0	>99.9999



Durable Antimicrobial Functions

<i>E.coli</i>	Dilution Ratio of Bacteria Solution after Exposure Time of 1.5 hr				Reduction of Bacteria (%)
	10	10 ²	10 ³	10 ⁴	
Blank	∞	∞	∞	44	-
BP-0 or RB-0	∞	∞	132	16	-
BP-4	∞	9	1	0	99.32
RB-4	11	4	0	0	99.70

Samples were exposed to day light for 40 consecutive days

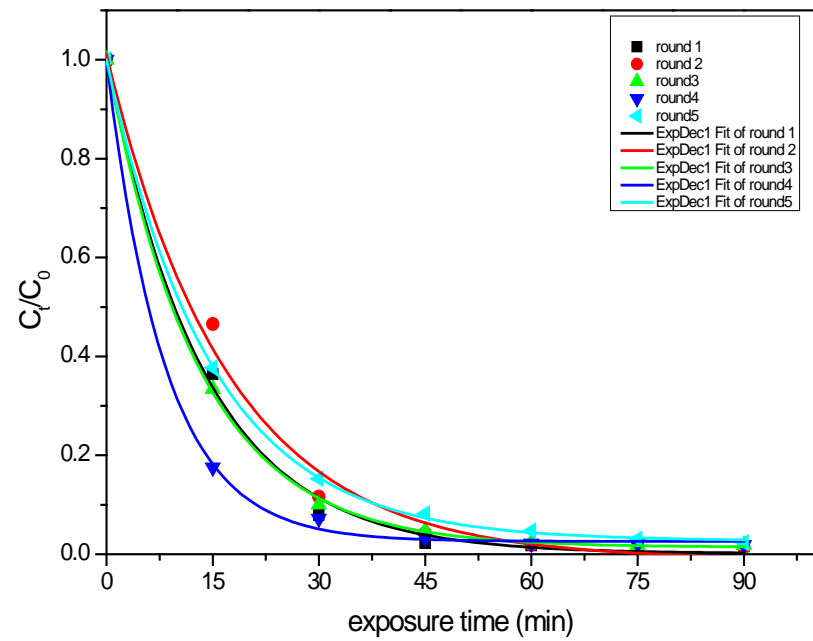
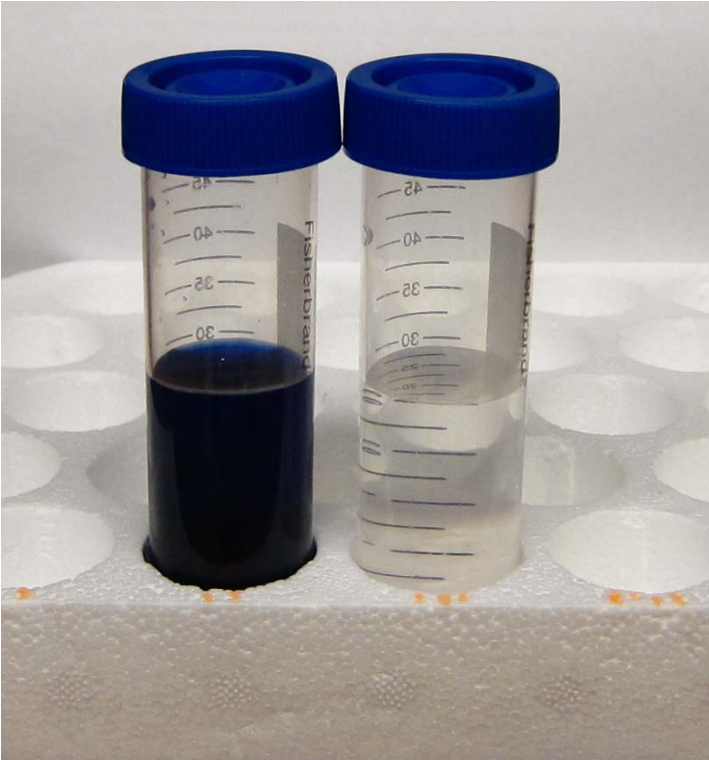


Durability against Abrasion

<i>E. coli</i>	Dilution Ratio of Bacteria Solution after Exposure Time of 1.5 hr				Reduction of Bacteria (%)
	10	10 ²	10 ³	10 ⁴	
Blank	∞	∞	∞	32	-
BP-0 or RB-0	∞	∞	76	6	-
BP-4 (0)	0	0	0	0	>99.9999
BP-4 (20)	0	0	0	0	>99.9999
BP-4 (100)	0	0	0	0	>99.9999
BP-4 (500)	0	0	1	0	98.68
BP-4 (1000)	0	0	4	0	94.74
RB-4 (0)	0	0	0	0	>99.9999
RB-4 (20)	0	0	0	0	>99.9999
RB-4 (100)	0	0	0	0	>99.9999
RB-4 (500)	0	0	0	0	>99.9999
RB-4 (1000)	0	0	0	0	>99.9999

Numbers in parenthesis were abrasion

Decolorization



Conclusions

- ◆ Several photo-sensitizers showed proper antibacterial functions on different textile materials including cotton, nylon, and protein fibers.
- ◆ Three anthraquinone dyes also showed production of reactive oxygen species under UVA exposure
- ◆ Polymer surface containing these photo-active structures could provide durable antimicrobial functions



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Thank You!

