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니코틴 분해 세균에 관한 연구(2)
-니코틴 분해 세균의 최적 생장조건 연구-

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Study on the Nicotine-Degrading Bacteria(2)
-The Optimal Growth Condition of Nicotinophiles-

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초 록

앞의 실험에서 선별된 34菌株의 니코틴 분해세균 중에서 Pseudomonas putida로 동정된 Strain NCT 27과 Arthrobacter oxydans biotype xanthum으로 동정된 strain NCT30에 대해서 이들의 니코틴 분해를 위한 최적 배지조건 및 그 밖의 생장특성을 조사하여 다음과 같은 결과를 얻었다.

니코틴 분해를 위한 최적배지조성은, KH_2PO_4 2.0gr, KCl 5.0gr, $MgSO_4 \cdot 7H_2O$ 20mg, $MnSO_4$ $6H_2O$ 0.2mg, $FeSO_4 \cdot 7H_2O$ 1.0mg, Co^{++}(Cobalt Acetate) 2.0γ Ni^{++} ($NiSO_4 \cdot 6H_2O$) 0.5γ, yeast extract 80mg/liter 이고 최적 초기 니코틴 농도는 Pseudomonas가 0.4%, Arthrobacter는 0.1%이었다. 그리고 최적생장온도는 두 경우 모두 30℃, PH는 7.0이며 배양중의 pH변화는, Pseudomonas는 대수생장기에서 산성으로 기울었다가 24~40시간 후에 염기성으로 변화하는데 비해 Arthrobacter는 전 기간 중 pH가 거의 일정하게 유지되었다.

니코틴에 대한 저항성은 Arthrobacter가 0.7%이상의 농도에서 생장이 완전히 저해됨에 비해 Pseudomonas는 1.0%까지 생장 가능하며 또한 담배추출물에서나, Nicotine 外의 다른 탄소, 질소 영양원이 포함되어 있는 배지에서도 니코틴을 효과적으로 분해할 수 있다. 그리고 최적배지조건에서 최대 니코틴 분해도는 각각 1.22g/hr/liter 및 0.186g/hr/liter이었다.

ABSTRACT

Among the 34 strains of Nicotinophiles selected in the previous experiments, strain NCT27 identified with *Pseudomonas putida* and strain NCT30 identified with *Arthrobacter oxydans* biotype *xanthum* were investigated for optimization of growth conditions

for nicotine degradation and other cultural characteristics.

The compositions of optimized medium were to be following: KH_2PO_4 2.0gr, KCl 5.0gr, $MgSO_4.7H_2O$ 20mg, $MnSO_4.6H_2O$ 0.2mg, $FeSO_4.7H_2O$ 1.0mg, Co^{++} (Cobalt Acetate), 2.0γ, Ni^{++} ($NiSO_4.6H_2O$) 0.5γ, and yeast extract 80mg per liter. The optimum initial concentrations of nicotine for growth were 0.4% for *Pseudomonas* and 0.1% for *Arthrobacter*, respectively. The optimum temperature and pH were 30°C and 7.0 for both of strains. The pH of culture medium of *Pseudomonas* was changed from acidic condition to basic one in going from the logarithmic growth phase to the stationary growth phase. In contrast with *Pseudomonas*, it remained constant in case of *Arthrobacter*.

The growth of *Arthrobacter* was completely inhibited in the nicotine concentration of 0.7%. However, *Pseudomonas* could grow even in the nicotine concentration of 1.0%. Moreover, it could grow successfully in the tobacco extract media as well as media containing carbon and nitrogen sources other than nicotine.

The maximum rates of nicotine degradation were to be 1.22 gr./hr./liter for *Pseudomonas* and 0.186 gr./hr./liter for *Arthrobacter*, respectively.

Ⅰ. 서 론

니코틴 분해세균의 서식지별 분포와 니코틴분해력이 우수한 34균주의 생물적 특성에 대해 앞서 보고한 바 있다.[7] 본 연구에서는 세균의 니코틴분해를 위한 최적조건을 알아보기 위해서 34균주 중 니코틴 분해력이 가장 우수하고 잎담배에 처리하기에 적합하다고 판단된 Strain NCT 27과, Strain NCT30을 선정하여 여러가지 배양조건이 세균의 생장과 니코틴 분해력에 미치는 영향, 그리고 잎담배에 적용하기 위한 적합성을 검토해 보고자 한다.

Ⅱ. 재료 및 방법

1. 균주

사용된 균주는 본 실험실에서 분리한 Pseudmonas putida NCT27과 Arthrobacter oxydans biotype xanthum NCT30이다.

2. 배지

금속이온에 대한 효과조사를 위한 배지는 이온교환수지를 통과시킨 증류수를 사용하여 1 liter당 nicotine 3.0ml, KH_2PO_4 2.0g, KCl 5.0g, yeast extract 0.1g의 기본조성에 각각의 금속염을 농도별로 첨가하여 제조하였으며 그밖의 실험을 위해서는 Sguros 배지에서 니코틴 농도와 pH만 달리하여 제조한 다음 500ml Erlenmeyer flask에 100ml씩 나누어서 121℃, 15Lb, 15分간 멸균하여 사용하였다.

3. 최적배지 조성 및 생장특성조사

평판배지에서 24시간 배양한 세균을 배양기당 10^6~10^7cell씩 접종한후 gyrotary shaker (New Brunswick Scientific Co. Model G-25)에서 196 r.p.m/min로 48시간 진탕배양하면서 6시간마다 균체밀도와 잔여니코틴 농도 및 pH 변화를 측정하여 생장상태를 조사하고 이에 따른 최적조건을 구하였다.

Ⅲ. 결과 및 고찰

선정된 두 균주 중 Strain NCT27은 앞의 실험에서 Pseudomonas putida로 동정되었으며, Strain NCT30은 앞의 보고서에서 분류한group Ⅰ의 Arthrobacter에 속하는 것으로써 운동성

이 없고 배양단계에 따라 다양한 형태를 가지는 것이 특징이다. 그리고 니코틴이 포함된 배지에서 20여시간 이상 배양하면 수용성의 푸른 색소가 생성되며 48시간 이상 지나면 색소는 갈변된다. 이때 배양액에 glucose를 첨가하거나 pH를 산성으로 낮추면 색소는 더욱 짙어진다. 이 균주는 Coussirat[1]가 보고한 Strain 11 S와 같은 생물적 특성을 가지는 것으로 판단되어 Sguros[9], Hylin[6]에 의해 보고된 바 있는 Arthrobacter oxydans biotype xanthum으로 동정하였다.

그림 I은 균체밀도와 건조균체 무게와의 관계를 나타내는데 Pseudomonas가 Arthrobacter에 비해 수율이 다소 높다.

표 1에서 배양액내의 초기 니코틴 농도가 세균의 생장 및 니코틴 분해력에 미치는 효과를 보았는데 Pseudomonas는 0.1~0.4% 농도에서 배지내의 니코틴을 95%이상 분해하며 균체생성은 0.4%농도에서 최대이다. 그리고 1.0% 니코틴 농도까지 생장가능하여 24시간내에 50%이상의 니코틴을 분해할 수 있다. 内田節子[10]가 분리한 Achromobacter nicotinophagum이 0.5%의 니코틴 농도에서 생장이 저해됨에 비해 이 균주는 니코틴에 대한 저항성이 매우 높은 균주

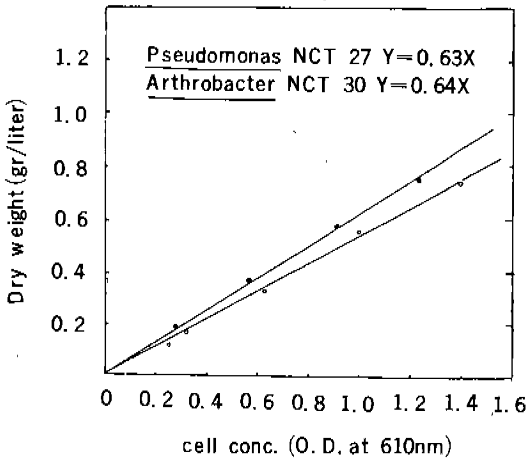

Fig. 1. Relations between cell concentration and dry weight;
●------● Pseudomonas NCT 27
○------○ Arthrobacter NCT 30

로 생각된다. 그러나 Arthrobacter의 경우에는 0.4% 이상에서는 니코틴 분해력이 미약하며 0.7% 이상에서는 생장이 완전히 저해된다. 이것은 두가지 균주가 서로 다른 니코틴 대사 경로를 가져서 Arthrobacter속에 속하는 세균은 니코틴을 부분적으로 분해하지만 Pseudomonas속

Table. 1. The Effects of Initial Concentration of Nicotine on the Growth of bacteia and Nicotine Degradation.

strain \ nicotine content (%)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Pseudomonas NCT 27										
O.D	0.520	0.635	1.075	1.115	0.575	0.596	0.720	0.740	0.660	0.644
R.N (mg/ml)	2.5	8.0	14.0	16.0	125.0	198.0	235.0	314.0	387.0	438.0
R.R (%)	97.5	96.0	95.0	96.0	75.0	66.8	66.5	60.7	57.3	56.2
Arthrobacter NCT 30										
O.D	0.965	0.690	0.360	0.365	0.310	0.225	N.G	N.G	N.G	N.G
R.N (mg/ml)	10.0	63.0	144.0	296.0	420.0	546.0	*	*	*	*
R.R (%)	90.0	68.5	52.0	26.0	16.0	9.0	*	*	*	*

Abbreviation : O.D=optical density
R.N=residual nicotine content
R.R=reduction ratio
N.G=no growth
* =not determined

의 경우에는 니코틴을 maleic acid 혹은 fumaric acid로 전환시켜서 체구성물질로 이용한다는[2]사실로 설명되어 진다.

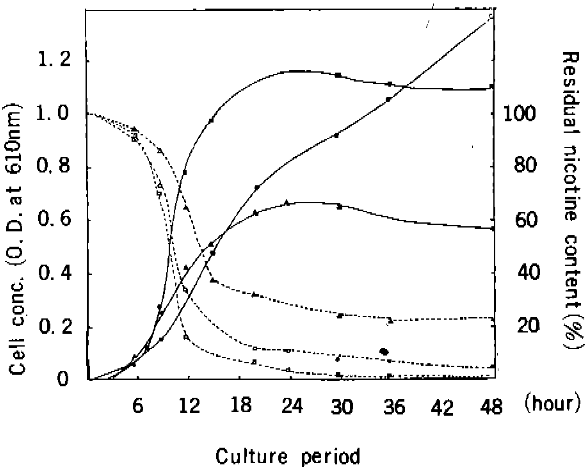

Fig. 2. The effects of initial concentration of nicotine on the growth (straight line) and nicotine degradation (dot line) of Pseudomonas NCT 27.

Symbols; ■——■ □——□ 0.2%, ●——● ○······○ 0.4%

▲——▲ △······△ 0.6%

그림 2는 Pseudomonas NCT27에 대해서 배양액 내의 초기 니코틴 농도에 따른 생장상태를 비교해 본 것인데 24시간이 지나면 생장이 정체되고 배지의 니코틴이 고갈됨을 알 수있다. 그리고 감소된 니코틴의 대부분은 6-12시간 사이에 소모된 것임을 알 수있다. 그러므로 대수생장기에 이른 세균을 접종한다면 6시간 이내에 배지내의 니코틴을 거의 소모할 수있을 것이다.

그림 3은 pH의 영향인데 두 균주가 모두 pH 7.0에서 니코틴분해력이 최대이지만 Arthrobacter NCT30의 경우 균체밀도는 pH8.0에서 최대로 나타났는데 이것은 pH변화에 따라 생성되는 색소의 정도가 달라서 균체밀도 측정에서 영향을 주었을 가능성도 있다. Coussirat[1] 등의 미동정된 세균에 대한 실험결과와 비교하면, 균주에 따라서 최적 pH가 5~8사이로 다양하게

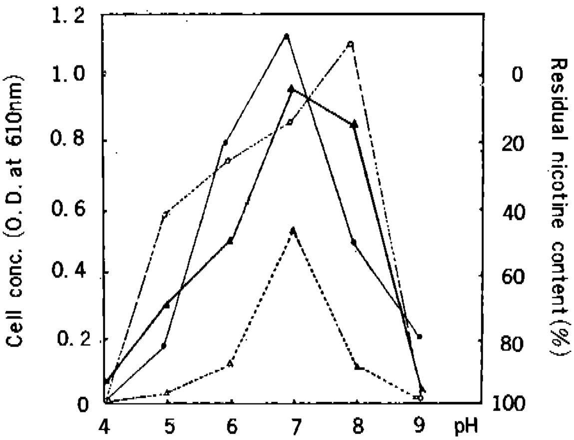

Fig. 3. The effects of pH on the growth (circle) and nicotine degradation (triangle); Symbols straight line Pseudomonas NCT 27 dotted line Arthrobacter NCT 30.

나타나 있는데 세균이 니코틴을 분해하는데는 초기 pH가 너무 높으면 저해가 된다는 보고도 있다.[2] 그런데 보통 건조가 끝난 잎담배의 pH는 5~6이므로 세균을 잎담배에 처리하고자 할 때는 잎담배의 pH와 비슷한 수준에서 잘 자랄 수 있는 세균이 이점을 가질 것이다.

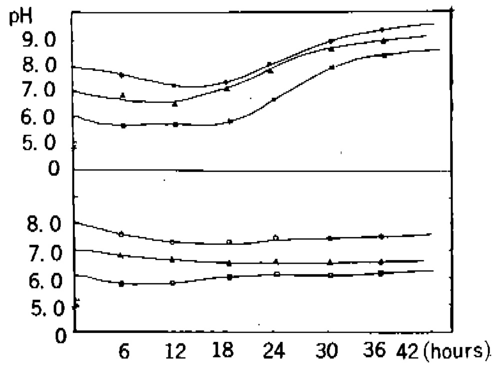

Fig 4. pH change of Pseudomonas NCT 27 (above) and Arthrobacter NCT 30 (below) in the different initial pH.

그림 4는 최적 pH 범위내에서 배양시간의 경과에 따른 pH 변화를 본 것인데 Pseudomonas NCT27은 대수생장기에서 pH가 약간 산성으로 기울어졌다가 다시 말기에는 염기성으로 변화한다. Hinz[5]에 의하면 Pseudomonas는 대수

생장기 이후에 니코틴의 pyridne ring의 질소중 일부가 암모니아로 변환되기 때문에 pH가 높아진다고 한다. 그러나 Arthrobacter NCT 30의 경우 pH가 거의 일정하게 유지되는데 이러한 사실도 두 균주가 니코틴을 분해하는데 있어서 서로 다른 중간대사과정을 거친다는 것을 뒷받침해 준다.

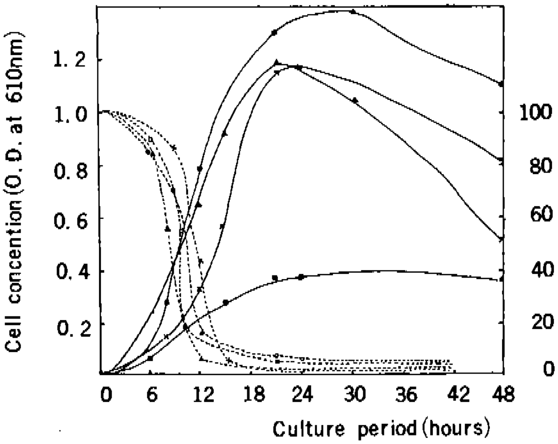

Fig. 5A. The effect of temperature on the growth (straight line) and nicotine degradation (dot line) by Pseudomonas NCT 27;
×——×25°C ●——●30°C ▲——▲37°C
■——■40°C

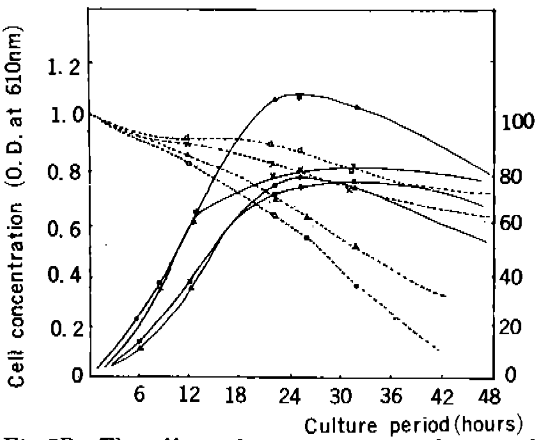

Fig. 5B. The effect of temperature on the growth (straight line) and nicotine degradation (dot line) by Arthrobacter NCT 30;
×——×25°C ●●——30°C ▲——▲37°C
■——■40°C

온도에 의한 영향을 보면 그림 5 A에서 Pseudomonas NCT27은 균체밀도는 30℃에서 최대이지만 니코틴 분해력은 25°～40℃에서 비슷하다. 이것으로 온도가 균체생성에 영향을 주지만 생장가능한 온도범위에서 0.3%농도의 니코틴은 이 균주에 의해 쉽게 분해됨을 알 수있다 그림 5 B에서는 Arthrobacter NCT30의 경우 균체생성정도에 따라 니코틴 분해력에 미치는 영향을 볼수있는데 역시 30℃가 최적온도로 나타났다.

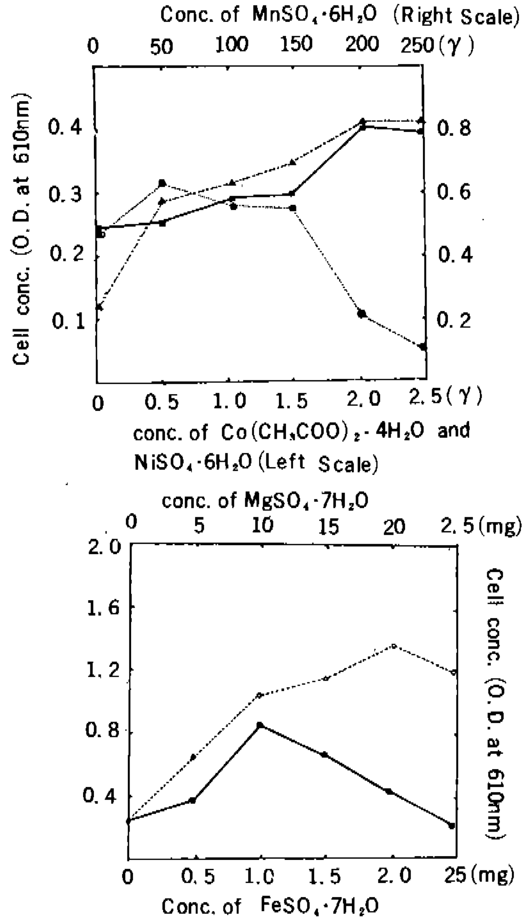

Fig. 6. The effects of metallic catalysts on the growth of Pseudomonas NCT 27·
○------○ $MgSO_4 \cdot 7H_2O$, ▲—·—▲ $MnSO_4 \cdot 6H_2O$,
●——● $FeSO_4 \cdot 7H_2O$,
■——■ co $(CH_3COO)_2 \cdot 4H_2O$
⊗······⊗ $NiSO_4 \cdot 6H_2O$

그림6에서 금속염들이 세균의 생장에 미치는 영향을 보았는데 $MgSO_4 \cdot 7H_2O$ 2.0mg, $MnSO_4 \cdot 6H_2O$ 0.2mg, $Co(CH_3COO)_2 \cdot 4H_2O$ 2.0ℓ 에서 최대 생장을 보이며 그 이상이면 약간 감소된다. 그리고 $FeSO_4 \cdot 7H_2O$는 1.0mg, $NiSO_4 \cdot 6H_2O$는 0.5γ에서 최적이며 각각 2.5mg, 1.5γ이상 이면 오히려 생장이 저해된다. Frankenburg[4]에 의하면 Mandeleeff periodic table의 Series 4에 속하는 원소들 중에서 Mn, Fe, Co, Ni 이 발효과정에서 촉매제나 촉진제로 작용한다고 한다. 그러므로 발효조건에서 안정성을 가지면서 수용성인 금속염들을 잎담배에 효과적으로 침투 시킬수있다면 잎담배의 산화를 촉진시킬 수 있을 것이다.

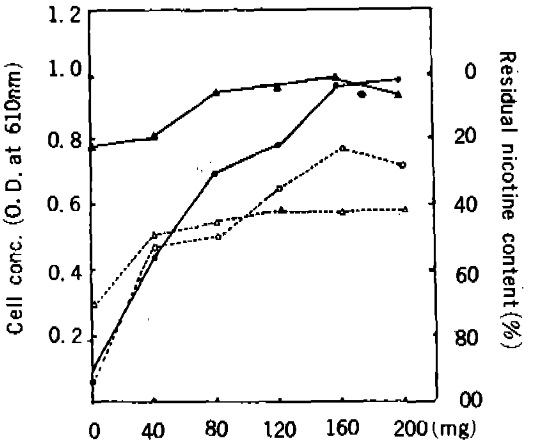

Fig. 7. The effects of yeast extract on the growth (circle) & nicotine degradation (triangl); straight line, Pseudomonas NCT 27 and dot line Arthrobacter NCT 30.

그림 7은 yeast extract의 영향을 본 것인데 농도가 커질수록 균체량은 증가하지만 니코틴 분해율은 80mg/liter의 수준에서 그 이상은 큰 차이가 없다. 이상의 결과에 의해 조성된 최적 배지 조건에서 배양시간에 따른 건조균체량과 잔여니코틴 농도와의 관계는 그림 8에서 나타낸바와 같은데 대수생장기에서 니코틴 분해도는 Pseudomonas NCT27이 1.22g/hr./liter이고 Arthrobacter NCT30은 0.186g/hr/liter이다.

표 2에서 니코틴 외의 다른 탄소, 질소 영양원을 배지에 첨가했을때 세균의 생장과 니코틴 분해력에 미치는 영향을 보면 Nitrite 화합물을 제외한 다른 화합물들이 첨가되면 균체량이 승증가되며 특히 C, N이 같이 포함되는 Na-glutamate, peptone을 첨가했을 때 크게 증가하는 것으로 보아 이 두 균주가 모두 Nicotine 外의 다른 영양원을 같이 이용할 수 있는 것으로 생각된다.

그림 9에서 glucose와 니코틴이 함께 포함된 배지와 니코틴만을 포함하는 배지에서 니코틴분해속도를 비교해 보았는데 Pseudomonas NCT 27의 경우 니코틴 분해도가 거의 같은것으로 보

Table. 2. The Effects of Carbon and Nitrogen Sources on the Growth of Bacteria and Nicotine Degradation.

C & N sources / strain	nicotine	nicotine + glucose	nicotine + $(NH_4)_2SO_4$	nicotine + KNO_3	nicotine + $NaNO_2$	nicotine + Na-glutamate	nicotine + peptone	glucose + $(NH_4)_2SO_4$
Pseudomonas NCT 27								
O. D	1.68	1.92	1.87	1.99	N. G	2.07	2.48	N. G
R. R(%)	94.5	95.0	93.0	95.0	*	92.0	87.0	*
Arthrobacter NCT 30								
O. D	1.32	1.84	1.65	2.15	N. G	2.75	4.80	N. G
R. R	55.0	88.0	53.0	90.0	*	56.0	68.0	*

In these experiment, 0.2% of each compounds were added in addition to the Sguros' medium.

Abbreviation : O. D = optical density at 610nm

R. R = reduction ratio

N. G = no growth

* = not determined

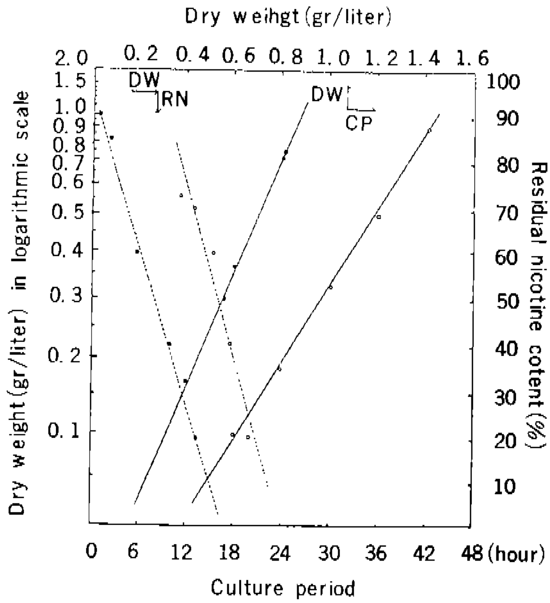

Fig. 8. The relationships among the cultureperiod, dry weight of cells and residual nicotine concentration;
●------● Pseudomonas NCT 27,
○------○ Arthrobacter NCT 30.

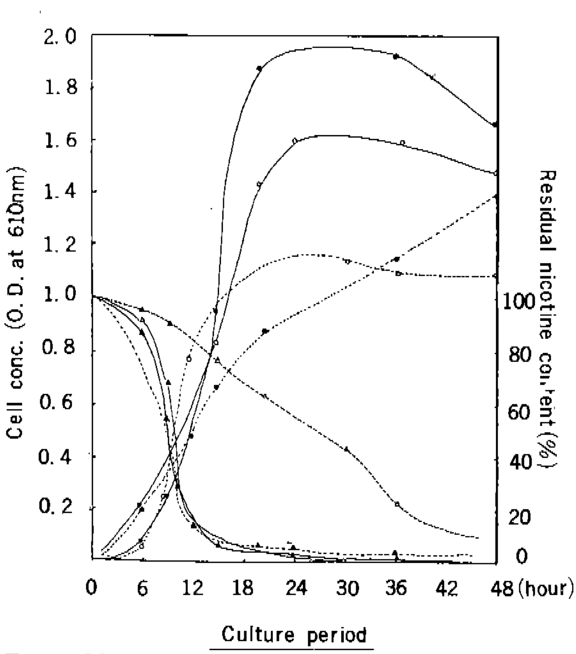

Fig. 9. The influence of glucose on the growth (circle) and nicotine degradation (triangle);
Symbols; straight line, Pseudomonas NCT 27
dotted line, Arthrobacter NCT 30
●------● glucose 0.2%+nicotine 0.2%
▲------▲
○------○ nicotine 0.2%

아 니코틴이 우선적으로 이용된다고 볼 수 있으며 Arthrobacter NCT30은 glucose가 있으면 탄소 영양원으로는 glucose를 우선적으로 이용하고 니코틴은 질소 영양원으로만 일부 이용되는 것으로 추측되는데 이것에 대해서는 다른 실험적 증거가 필요하다.

Decker[3]에 의하면 Arthrobacter는 니코틴 대사과정에서 니코틴외의 다른 탄소, 질소 영양원이 함께 존재하면 니코틴 산화효소의 작용이 일부 억제되어 니코틴이 부분적으로 분해된다고 한다.

표 3은 Pseudomonas NCT27에 대해서 잎담배에 대한 적응성을 조사한 것인데 평판 배지에서는 니코틴이 0.15~0.54% 포함되어 있을때 생장이 잘 되었으며 니코틴 분해력은 Sguros[9] 배지를 쓸 때에 비해 50~70%정도된다. 이것은 잎담배에는 니코틴이 여러가지 화합물의 형태로 존재하기 때문에 시약을 사용할 때와는 차이가

Table. 3. Growth of Pseudomonas NCT 27 in the Tobacco Extract Media.

media	amount of powdered tobacco (%)	2.0	4.0	8.0	12.0	16.0	18.0	* 8.0 (in D. W)
Broth	nicotine content (%)	0.08	0.15	0.31	0.46	0.54	0.61	0.31
	reduction ratio (%)	*	71.0	53.0	45.0	37.0	*	17.0
Agar	growth	±	+	++	++	+	−	+

* Tobacco powder was prepared from Hyangcho tobacco leaves and added to Sguros' media.

있는 것으로 생각된다. 그리고 같은 조건에서 배지대신 증류수를 쓰면 니코틴 분해력은 53.0%에서 17.0%로 감소되는데 이것으로서 적당한 조성의 배지가 세균의 활성에 미치는 영향을 알 수있다.

이상의 결과에서 최우수 균주로 선정된 Pseudomonas NCT27은, 식물체나 인체에 대해 비병원성이며 냄새, 색소가 없고 담배추출물에서 잘 자라며 고농도의 니코틴에 대해 저항성을 가진다. 그리고 다른 영양원보다 니코틴을 선택적으로 빠르게 이용하므로 당이 적은 Burley, 하급엽의 니코틴 감소를 위해 효과적으로 이용할수있으리라 생각된다. 그러므로 앞으로 잎담배내에서의 생육, 발효조건에 대해 연구되고 또한 균체의 이화학적 특성및 니코틴 대사 중간산물이 규명된다면 산업적으로 유용하리라고 생각된다.

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The Factors Affecting Filling Power of Tobacco Shreds and Cigarette Hardness.

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ABSTRACT

The factorial experiments were carried out to observe the effects contributed by three factors, i.e. moisture content, cigarette weight and length of the tobacco shreds on the cigarette hardness. The effect of the length of tobacco shred on the filling power was also investigated.

The factorial effects contributed by moisture content, cigarette weight and length of tobacco shred on the cigarette hardness of Hicks tobacco were 48.6-51.3%, 39.8-43.2% and 3.6-5.6%, respectively. However, the factorial effects of Burley tobacco for the corresponding factors were 56.9-58.7%, 27.3-37.1% and 2.9-8.1%, respectively.

The variation rates of filling power due to the shortening of shred length were differently changed by each moisture level. However, at constant moisture content (12%), it has a range of 8-12% in average for each tobacco.

Introduction

The hardness and the filling power have been considered to be the most important physical properties of cigarette and cut tobacco in the view point of the cigarette manufacturer. It has, therefore, been studied intensively by many workers, since the changes of these properties are critical to the quality, consumer preference, smoking taste and the results of quality analysis. It is also an important factor to be controlled in the stability of the ends of cigarette during the packing processes in the factory and marketing.

It has been reported that cigarette hardness is affected by the weight of tobacco rolled into the specified volume of cigarette, and by moisture as well as filling power (4-9, 18, 22).

In addition to these factors, the cut width and the stalk position of tobacco also affect the cigarette hardness (3,7).

On the influencing factors for the filling power of tobacco, Pietrucci (14-16) et al. have pointed out that the filling power of tobacco is substantially dependent upon the length, thickness and cut width. Many other workers have also reported the influence of moisture and temperature on the filling power (4-6, 11-13, 19-21).

However, we can assume that cigarette hardness will be varied by some influencing factors as those factors are at work compositely at once. In this circumstances, it will be very effective to control the influencing factors for the better quality of cigarette if we will be able to describe quantitatively the factorial effect influenced by the respective factor.

The factorial experiments of three-way layout were conducted to analyze the effects contributed by three factors (moisture, weight of cigarette and length of tobacco) and by three levels for each factor such as these factors and levels having an effect compositely on the cigarette hardness.

The influences of the shred length and moisture on filling power were also observed to describe their relation to the cigarette hardness.

Materials and Methods

The factors and levels, and the design of the factorial experiment of three-way layout for this study were controlled like Table 1 and Table 2.

Tobacco leaves used for these studies were prepared with the standard group of leaf and cutter 3rd grade from two varieties of Hicks (HsB3, HsC3) and Burley (BrB3, BrC3) grown in 1979 in Korea.

Table 1. The controlled factors and levels of factorial experiments for cigarette hardness

Factor	Level		
Length of shreds (mm) (sieve size)	$L_1=4.76$	$L_2=2.38$	$L_3=1.00$
Moisture content(%)	$M_1=10$	$M_2=12$	$M_3=14$
Weight of cigarette (mg)	$W_1=800$ (1) 850 (2)	$W_2=900$ 950	$W_3=1000$ 1050

(1) Weight of Burley Cigarette.
(2) Weight of Hicks Cigarette.

Table 2. Three-way layout of factorial experiments in combination with the factors and levels

L. / M.C / W.	L_1			L_2			L_3		
	M_1	M_2	M_3	M_1	M_2	M_3	M_1	M_2	M_3
W_1	111	121	131	211	221	231	311	321	331
W_2	112	122	132	212	222	232	312	322	332
W_3	113	123	133	213	223	233	313	323	333

For the preparation of testing sample, tobacco leaves were stripped by hand. The hand stripped tobaccos were cut in 0.9mm of cut width on a MM-3 cutting machine. Preparatory to classify the tobaccos into different sizes, each sample of cut tobacco was conditioned to have approximately 12% of moisture, then the tobaccos were sieved by the sieve shaker (Model SPA, Mitsubishi, Japan) nested 9.52, 5.66, 4.76, 2.38, 1.00 and 0.5 millimeter of the standard sieves during 30 seconds of sieving time with the speed of 180 r.p.m.

The classified cut tobaccos were then divided into two groups. One group contains all of six classified tobaccos for measurement of filling power, and the other group contains only 4.76, 2.38, and 1.00 milimeter to make a plain testing cigarette in a different level of cigarette weight for the measurement of cigarette hardness.

For the measurement of filling power, several different ranges of moisture levels for each length of tobaccos, were obtained through further conditioning. For these processes, each sample was divided again into several lots and was conditioned at 25°C and at 35, 41, 46, 49, 55, 60, 64, 73, 79, 85 and 90 per cent relative humidity(RH) for one week in a desiccator to obtain a different range of moisture level for each lot. The filling power of each lot was measured by using a densimeter (Heinr. Borgwaldt, Hamburg, West Germany). The moisture content was determined at time of filling power measurement by drying samples in a drying oven for 3 hours at 100°C.

The cigarettes for measuring hardness were prepared with the various levels of weight and three different length of cut tobacco (4.76, 2.38, 1.00mm). The circumference of cigarettes was first selected to be 25.1±0.1mm (diameter 7.99±0.03mm) by using a Filtrona circumference tester.

Preparatory to classify the cigarettes into different weight levels, the circumference uniformed cigarettes were preconditioned at 25°C and at 65 per cent RH for one week in an environmental chamber (Model 3950, Forma Scientific, U.S.A) to minimize the variation of cigarette weight due to the difference of moisture content among the samples. The weight screening was carried out by an electronic reading balance (ED-200 MO, Shimadzu, Japan). Three different weight levels of cigarettes (800, 900, 1000mg) were exactly chosen from the cigarettes made by Burley tobaccos. In the case of Hicks cigarettes, another weights (850, 950, 1050 mg) of cigarettes were used to meet the conditions of Table 1. The sorted simples were again divided into four lots. Each lot was conditioned in the same desiccator at 25°C and at 60, 64, 69, and 73 per cent RH for one week to obtain different ranges of moisture level. The same densimeter mentioned above was used for the measurement of cigarette hardness.

Cigarette hardness in this report was expressed as follows,

$$H = D_1 - D_2 = \Delta d \qquad (1)$$

where H denotes cigarette hardness, D_1 and D_2 indicate the diameter of cigarette before and after compression. Therefore, lower value of H means harder cigarette.

The regression equations were obtained to describe the variation of filling power in terms of moisture levels for each size of shredded tobaccos. The variation of cigarette hardness caused by the controlled factors was also analyzed by means of the analysis of variance to explore the factorial effect of each factor.

Results and Discussion

- Filling power

The general trend of the variation rates of filling power was revealed to be small at high moisture levels in contrast with the one at low moisture contents.

The observed rates of variation due to the changes in moisture content for Hicks tobaccos were to be 4.3-7.4% for

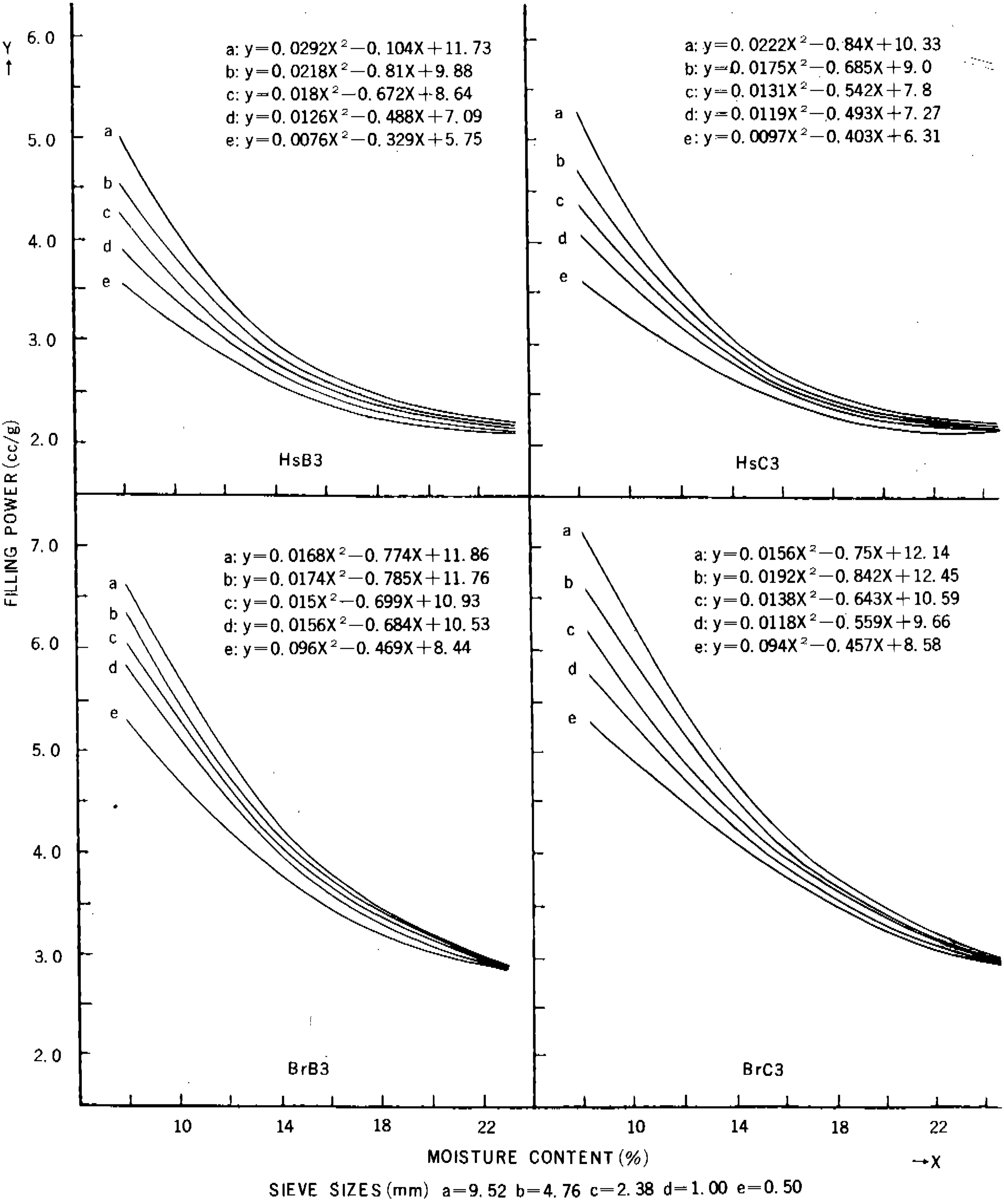

Fig. 1. Relation of filling power in shredded tobacco to moisture content and length of shreds,

the leaf third grade(HsB3), 4.6-7.7% for the cutter third grade(HsC3), respectively(Fig. 1). These rates of variation were appeared to be higher in going to longer length of shreds. This fact implies that the effect of moisture level can be greater in the long shreds than that of the short one.

When the length of shreds is changed, it is difficult to state the exact trend of filling power variations. Because it is quite substantially varied by each step of moisture level. However, at constant moisture content (12%), it has a range of 8-12% in average for each tobacco.

With regard to the Burley tobaccos, the same trend of variation as Hicks tobaccos were observed. The variation rates of filling power due to the changes in moisture content were to be 4.2-5.8% for the cutter 3rd grade(BrC3) and 4.6-6.1% for the leaf 3rd grade(BrB3), respectively.

The observed rates of variation on the change of shred length show us somewhat different features (Fig. 2). In the range of the decreasing ratio of sieve sizes less than 75%, as the length decreased from 9.56mm to 2.38mm, the decreasing ratio of filling power is observed to be a little (5-6%). However, when the sieve sizes decrease up to 90%, from 9.56mm to 1.00mm, the filling power decreases up to about 13%. Furthermore, when the sieve sizes are reduced to be 95%, from 9.56mm to 0.50mm, the drastical decrease of filling power (about 18%) can be observed.

There are also somewhat differences in the rates of variation between leaf grades and varieties. It seems to be quite dependent on the intrinsic characteristics of leaf tobacco.

The regression equation for each tobaccos is also derived from the observed values to predict the effects of changes in sieve sizes on the filling power (Fig. 2). According to these equations, one may be able to estimate the variation of filling power due to the change of shred length. These equations may also be effective in preventing the degradation of cut rag in the processes of cigarette manufacturing.

- Cigarette hardness

As cigarette weight is increased and the other parameters remain constant, there is an increase in hardness (Table 3). As moisture content increases, there is a great decrease in hardness. These similar results also have been reported by other workers (4-9, 18, 22).

With regard to the change in shred length, there is, however, small decrease in hardness as length decreases. This fact suggests that shred length has little effect on the cigarette hardness compare with the other two factors.

One can easily assume here that the variation of the cigarette hardness may be influenced by the composite factors simultaneously. The main object of this study is to see the factorial effect quantitatively and separately for each influencing factor.

The data shown in the Table 3 for each cigarette sample were treated by means of the procedures of the analysis of variance to observe each effect on the cigarette hardness.

The effects of the three main factors on the cigarette hardness were found to

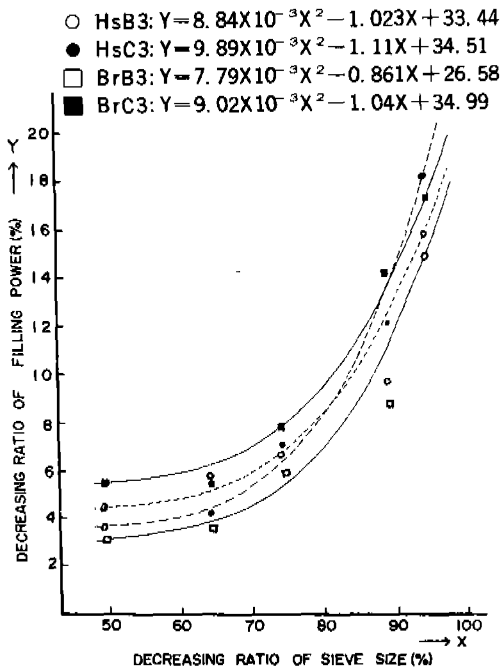

Fig. 2. Relation of decreasing ratio of filling power to that of sieve size at 12% moisture content.

be quite high significant (p>0.01). Nonetheless, interactions among the factors were negligible (Table 4).

The factorial effects contributed by moisture on the cigarette hardness of Hicks tobacco were to be 48.6% (HsB3)-51.3% (HsC3), and by cigarette weight, it appeared to be 39.8% (HsC3)X_2 43.2% (HsB3). However, the effects of tobacco length were only 3.6% (HsB3)X_2 5.6% (HsC3), respectively.

The factorial effects of Burley tobaccos of the corresponding factors were to be 56.9%X_2 58.7%, 27.3%X_2 37.1% and 2.9X_2 8.1%, respectively.

As can be seen from these results, it is clear that cigarette hardness has been affected mainly by the two factors, moisture and cigarette weight, about 90 per cent for all the tobaccos (Fig. 3). Be-

Table 3. Data of cigarette hardness obtained from the factorial experiments in combination with the factors and levels.

Sample	Length (mm) / Moisture (%) / Weight (mg)	4.76			2.38			1.00		
		10	12	14	10	12	14	10	12	14
HsB3	850	2.46	2.85	3.30	2.35	2.97	3.38	2.73	3.02	3.54
	950	2.05	2.57	2.84	2.24	2.58	2.93	2.34	2.79	3.08
	1050	1.78	2.08	2.47	1.82	2.20	2.71	1.95	2.26	2.60
HsC3	850	1.99	2.38	2.86	2.08	2.50	3.20	2.25	2.74	3.32
	950	1.40	1.86	2.59	1.46	2.17	2.75	1.82	2.33	2.89
	1050	0.98	1.45	1.93	1.17	1.66	2.12	1.29	1.73	2.43
BrB3	800	1.88	2.52	2.96	2.16	2.58	3.15	2.30	2.80	3.28
	900	1.62	2.22	2.63	1.70	2.18	2.70	1.82	2.28	2.83
	1000	1.25	1.71	2.24	1.35	1.76	2.36	1.60	1.97	2.30
BrC3	800	1.53	2.13	2.72	1.70	2.24	2.68	2.05	2.55	2.92
	900	1.21	1.82	2.25	1.45	1.80	2.39	1.67	2.23	2.47
	1000	1.08	1.50	1.96	1.29	1.75	2.01	1.51	1.78	2.18

Table 4. Analysis of variance of cigarette hardness originated by influencing factors

Sample	Factor	df	S	V	F_o	F'_o	S'	ρ(%)
HsB3	L	2	0.2567	0.1284	5.26**	*	0.2079	3.64
	M	2	2.8243	1.4122	57.88**	**	2.7755	48.55
	W	2	2.5185	1.2593	51.61**	**	2.4697	43.20
	L × M	4	0.0162	0.0041	–		–	–
	M × W	4	0.0349	0.0087	–		–	–
	L × W	4	0.0258	0.0065	–		–	–
	E	8	0.0409	0.0051	–		0.2642	4.62
	(E)	(20)		(0.0244)				
	Total	T 26	5.7173				5.7173	100
HcC3 s	L	2	0.6273	0.3137	142.59**	**	0.5635	5.64
	M	2	5.1882	2.5941	1179.14**	**	5.1244	51.29
	W	2	4.0387	2.0194	917.91**	**	3.9749	39.79
	L × M	4	0.0138	0.0035	1.59		–	–
	M × W	4	0.0661	0.0165	7.5 **		–	–
	L × W	4	0.0386	0.0097	4.41*		–	–
	E	8	0.0174	0.0022	–		0.3273	3.28
	(E)	(12)		(0.0057)				
	Total	T 26	9.9901				9.9901	100
BrB 3	L	2	0.2589	0.1295	6.05**	**	0.2161	2.91
	M	2	4.2731	2.1366	99.84**	**	4.2303	56.97
	W	2	2.7935	1.3968	65.27**	**	2.7507	37.05
	L × M	4	0.0277	0.0069	–		–	–
	M × W	4	0.0156	0.0039	–		–	–
	L × W	4	0.0288	0.0072	–		–	–
	E	8	0.0275	0.0034	–		0.228	3.07
	(E)	(20)		(0.0214)				
	Total	T 26	7.4251				7.4251	100
BrC3	L	2	0.5555	0.2778	55.56**	**	0.4887	8.13
	M	2	3.5955	1.7978	359.56**	**	3.5287	58.70
	W	2	1.7071	0.8536	170.72**	**	1.6403	27.28
	L × M	4	0.0457	0.0114	2.28		–	–
	M × W	4	0.0472	0.0118	2.36		–	–
	L × W	4	0.0207	0.0052	1.04		–	–
	E	8	0.0401	0.005	–		0.3541	5.89
	(E)	(20)		(0.0334)				
	Total	T 26	6.0118				6.0118	100

Level of significant * 0.05

** 0.01

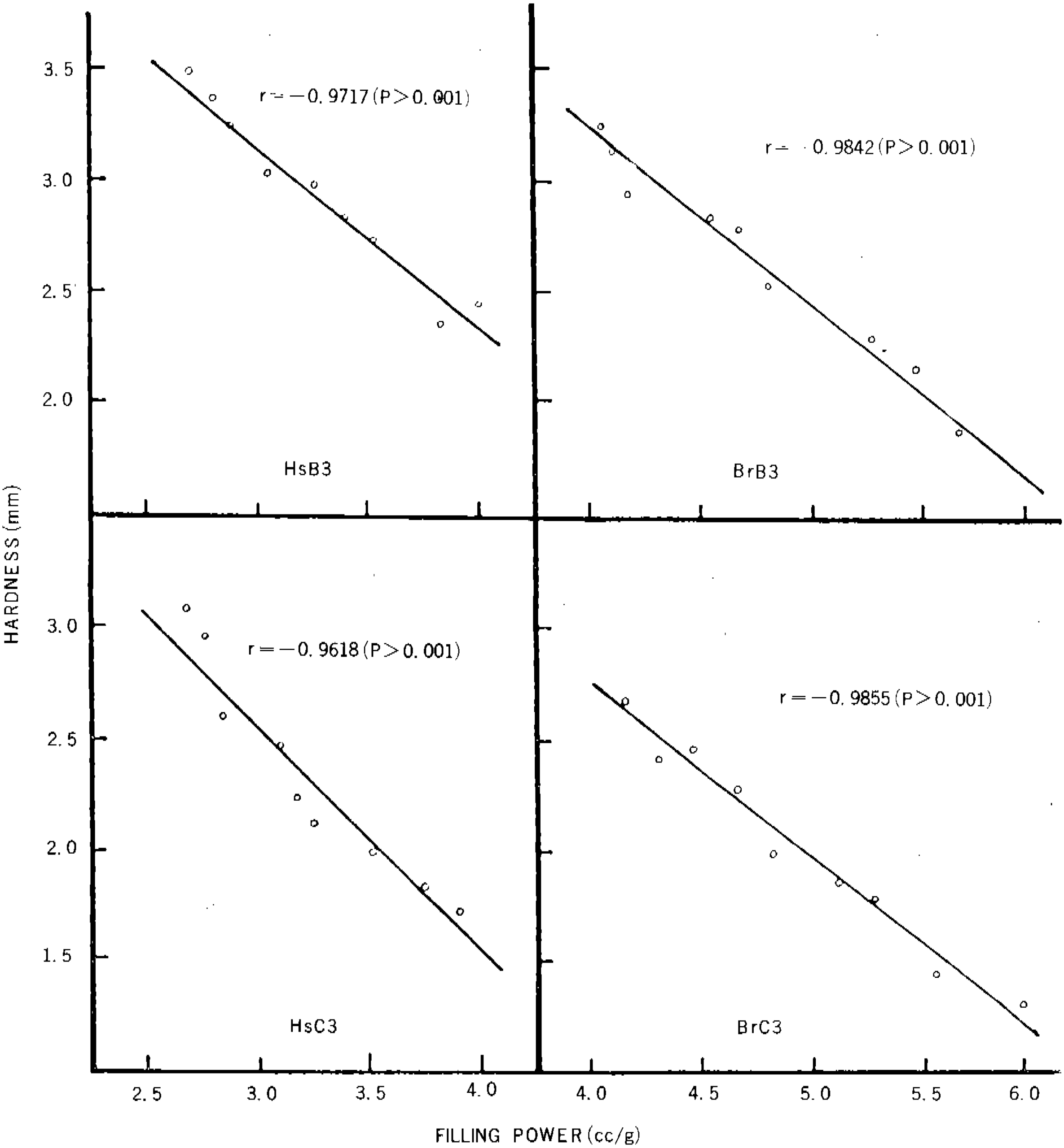

Fig. 4. Correlation between filling power and cigarette hardness.

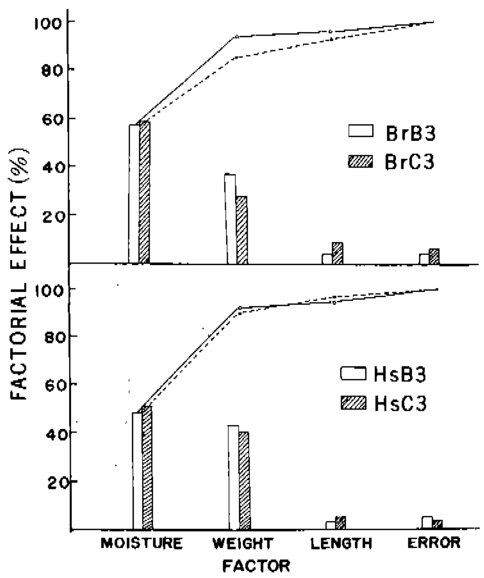

Fig. 3. Comparison of the effect of three factors on the cigarette hardness.

tween the two main factors, it is likely to say that moisture is more effective to the hardness than cigarette weight.

In order to predict the values of cigarette hardness, the multiple linear regression equation for each tobacco was derived from the experimental data among the influencing factors of moisture content(X_1), cigarette weight(X_2) and the length of shreds(X_3).

$$\text{HsB3}: Y = 4.1311 + 0.1797X_1 \quad 3.7389X_2 - 0.0542X_3 \qquad (2)$$

$$\text{HsC3}: Y = 3.6648 + 0.2681X_1 \quad 4.7556X_2 - 0.0901X_3 \qquad (3)$$

$$\text{BrB3}: Y = 2.9447 + 0.2456X_1 \quad 3.9389X_2 - 0.0439X_3 \qquad (4)$$

$$\text{BrC3}: Y = 2.2619 + 0.2233X_1 \quad 3.0611X_2 - 0.0836X_3 \qquad (5)$$

As can be seen, these equations now allow us to predict effects of change in up to three variables, at once. This predictive capability may be useful in the design of new products and in the manipulations of present one.

- Correlation between filling power and cigarette hardness.

The filling power of the tobaccos was highly correlated with corresponding value of cigarette hardness (Fig. 4). Other workers have also reported a high degree of correlationship between filling power and cigarette hardness (2, 3, 6, 22).

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